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BACTERIAL AEROSOLS FROM BURSTING BUBBLES

A THESIS

Presented to

The Faculty of the Graduate Division

by

Frederick Benjamin Higgins, Jr.

In Partial Fulfillment

of the Requirements for the Degree

Doctor of Philosophy in the School of Civil Engineering

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BACTERIAL AEROSOLS FROM BURSTING BUBBLES

Approved:

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Date approved by Chairman: May 13, 1964

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SUMMARY

Meteorologists have been aware for many years that bubbles bursting in the ocean produce aerosols that form condensation nucleii for cloud and fog formation. The mechanism of aerosol generation has been well characterized by several investigators, and this mechanism appeared to be capable of carrying microorganisms into the air from polluted waters. However, a review of the literature revealed very little information that would aid in evaluating the potential hazards of sewage or naturally occurring bubbles bursting in contaminated water.

Since the number of probable variables was large, the present study was confined to a laboratory investigation of the generation process, the response of a number of species of microorganisms to aerosolization, the effect of some chemical additives, and an estimation of the magnitude and effect of several major variables.

A wind tunnel was constructed approximately two feet wide, two and one-half feet high, and extending some 22 feet from an inlet window to an outlet window. Due to the size and shape of the laboratory, a 120° bend was installed downstream from the sampling zone. The tunnel was fitted with straightening tubes, a 74 liter aeration tank set into the floor, a variable speed, DC motor driven exhaust fan, and plexiglass windows and access ports. Bubbles were generated by pumping filtered air through fritted glass bubblers installed in the aeration tank.

Air samples were withdrawn through intake probes sized to approximate isokinetic conditions. Samples were connected to the intake outside the

tunnel to allow easy access, reduce contamination, and prevent velocity disturbances. The slit and Andersen samplers were employed in experiments reported, although all-glass impingers were used unsuccessfully in some preliminary work. Both samplers operate on the principle of impingment on an agar surface and allow the measurement of number of particles containing viable organisms.

Air was exhausted from the wind tunnel through a window to the outside air. Therefore, only non-pathogenic microorganisms could be employed. Since the coliform group of microorganisms is of traditional importance in sewage pollution, strains of Escherichia coli, Escherichia freundii, Escherichia aurescens, and Aerobacter aerogenes were chosen for evaluation. Serratia marcescens was selected as a species with similar response to the airborne environment as E. coli, but which has been more extensively studied. Streptococcus durans and Streptococcus salivarius were expected to show somewhat less loss in viability than the previously named species, while Bacillus subtilis spores were used as a reference species which was expected to be little affected by the aerosol production process.

It was found that while significant numbers of viable bacteria were recovered, the relative recoveries of the species used differed from those anticipated. S. marcescens concentrations were found up to five times those of B. subtilis under comparable conditions. Allowing for the expected loss in viability of S. marcescens due to dissication, the true difference in aerosol production rates may have been as great as 20. The coliform group, on the other hand, showed very low recoveries. Viable E. coli and E. freundii were not recovered, A. aerogenes was found only in large

unevaporated droplets, and E. aurescens was recovered in concentrations less than one per cent of those for B. subtilis.

Concentrations of S. durans and S. salivarius of 50 and 30 per cent respectively, of those for B. subtilis were in accordance with the expected resistance of these bacteria and tended to show that B. subtilis concentrations were valid standards for comparison. The abnormal behavior of S. marcescens was deduced from experimental evidence to result from concentration at the surface of the liquid. While it could not be shown with the apparatus employed, it is suspected that the coliform group showed very low generation rates because of a migration away from the surface. In prior comparisons by others of the survival of S. marcescens and E. coli in air, the two have been shown to be comparable. In the present study, a difference in relative viability at the end of 1.5 seconds of at least 500 was shown.

Actual numbers of aerosols produced per unit time under seemingly identical conditions were found to vary widely, especially with the vegetative species. The production rate was, however, shown to be related to the chemical composition of the aeration liquid, the stability of bubbles, the bubble size, and the rate of bubble production. Minor variations in surface tension had little effect, although the addition of one to five milligrams per liter of a detergent decreased aerosol generation. Aerosol production increased with concentration of bacteria in the aeration liquid up to 1.6×10^6 per milliliter, which was the highest value used. In the case of S. marcescens, the number found decreased with time of aeration, indicating a decreased resistance to drying.

Measured aerosol particle diameters varied with the concentration of solids present. However, with aeration liquids containing up to 260 mg/l total solids, large numbers of two to five micron diameter particles were found. The unevaporated droplets had a median diameter not greater than 110 microns and a geometric standard deviation of approximately 1.55. The smaller droplets were too small to arise from the jet-droplet mechanism and were attributed to particles thrown from bubble clusters.

The slit sampler was found to recover 18 per cent more viable bacterial particles than the Andersen. With either sampler, colony counts were decreased significantly when differential media were employed for the collection of coliform bacteria or the Streptococci.

The following are the conclusions resulting from this study.

1. The production of viable bacterial aerosols by bursting bubbles is highly dependent upon species. Using B. subtilis as a basis for comparison, excess numbers of S. marcescens, comparable numbers of the Streptococci and a pronounced deficiency of coliform organisms were noted. The data suggests that the non-uniform production of aerosols is due to a difference in concentration of the cells at the surface from that in the bulk of the aeration liquid. The difference may be either positive or negative and is highly dependent upon both species and composition of the aeration liquid.

2. The coliform group of bacteria may not be a reliable index of bacterial air pollution resulting from sewage aeration. The very low aerosolization rate of coliform organisms precludes the conclusion that

the absence of members of the group indicates an absence of pathogenic bacteria in air. Until information becomes available on the aerosolization of pathogens, it is suggested that a total colony count may be a more appropriate measure of air contamination by aeration processes.

3. Calculated original diameters of droplets produced by bursting bubbles indicated that many particles were too small to have originated through the jet-droplet mechanism. A possible source may be the thickened film at the point of juncture of adjacent bubbles.

4. In addition to the primary variables of species and composition of aeration liquid, other variables affecting the aerosolization process were surface tension, bubbling rate, bubble size, wind velocity, concentration of cells, and physiological state of the culture. The effects of air and water temperature or of relative humidity could not be isolated from the data. The large number of variables affecting the net aerosolization rate indicates that a realistic evaluation of the bacterial air pollution resulting from an aeration process should require large volume air samples taken over an extended period of time.

GLOSSARY OF ABBREVIATIONS

Aa	-	Aerobacter aerogenes.
Bs	-	Bacillus subtilis var. niger.
C	-	number of aerosols per cubic foot of air.
cfm	-	cubic feet per minute.
cm	-	centimeter.
C_o	-	number of aerosols per cubic foot air at the downstream sampling point.
C_V	-	coefficient of variation, or standard deviation divided by the mean.
C.L.	-	centerline.
$C_{4.1}$	-	number of aerosol per cubic foot at a wind velocity of 4.1 feet per second.
D	-	demineralized water.
DK	-	demineralized water plus 160 mg/l K_2HPO_4.
DKP	-	demineralized water plus 160 mg/l K_2HPO_4 and 100 mg/l peptone.
DP	-	demineralized water plus 100 mg/l peptone.
Ea	-	Escherichia aurescens.
Ec	-	Escherichia coli.
Ef	-	Escherichia freundii.
fps	-	feet per second.
H	-	height of sampling point above floor of tunnel.
K	-	a concentration of 160 mg/l K_2HPO_4 (when referring to aeration liquid)
K_1	-	aerosol concentration of a species divided by the concentration for B. subtilis under equivalent conditions.

lpm	-	liters per minute.
mg/l	-	milligrams per liter.
ml	-	milliliters.
mm	-	millimeters.
N	-	number of bacteria per ml of aeration liquid.
N_s	-	colony count of a single sample.
P	-	a concentration of 100 mg/l peptone (when referring to aeration liquid).
R	-	number of viable aerosol particles passing the downstream sampling point each second.
Sd	-	Streptococcus durans.
Sm	-	Serratia marcescens.
Ss	-	Streptococcus salivarius.
T	-	tap water.
TP	-	tap water plus 100 mg/l peptone.
μ	-	microns.
V	-	velocity.

CHAPTER I

INTRODUCTION

General

The production of small water droplets by air bubbles bursting in water has been known and studied by meteorologists for many years. Such droplets, arising from the sea, have been shown to produce salt nucleii which aid in cloud formation (1) and are suspected of providing the means of charge separation required for the generation of lightning (2). A. H. Woodcock, of the Woods Hole Oceanographic Institute, suggested to the sanitary engineering profession in 1955 (3) that aerosols arising from bursting bubbles in polluted waters might carry pathogenic organisms into the air. It was the purpose of the investigation reported herein to establish a basis for estimation of the hazard posed.

Review of Literature

Two publications were found which are concerned with bacterial aerosols arising from sewage treatment operations. Merz (4) reported the results of a study on airborne coliform organisms arising from a sewage aeration tank, the exhaust from a covered sedimentation basin, a hose discharging settled sewage, and a sprinkler discharging settled sewage. The objective of the investigation was to evaluate the hazard associated with sprinkling treated sewage on a golf course. Samples were collected downwind from potential aerosol sources using a rectangular impinger oriface discharging onto the surface of 300 ml of

0.2 per cent gelatin in water. The impinger tube and liquid were contained in a 2 liter flask. Known volumes of the sampler fluid were filtered through a membrane filter for determination of the coliform count.

Of the four sources sampled, coliforms were found only downwind from the sprinkler, and sufficiently close that spray could be felt. It was concluded that sewage sprinkling presented no hazard except that of direct contact with unevaporated droplets. However, as will be shown later, this conclusion is open to question because coliform organisms are more sensitive to the airborne state than many pathogens. A more realistic evaluation of the problem would have resulted from the determination of the increase in total number of microorganisms as shown by a wide-spectrum collection media.

The bacterial air pollution in the vicinity of a trickling filter sewage treatment plant was measured by Albrecht (5) in 1958. Samples were collected upwind and downwind from each component of the plant. Both the Wells centrifuge and the midjet impinger samplers were employed. Coliform determinations were made using Tergitol-7 agar and general bacterial counts were obtained on nutrient agar. Relatively few coliform organisms were recovered, but the nutrient agar counts showed an increase in concentration of from 2 to 144 organisms per cubic foot of air at the edge of the trickling filter, decreasing to 7 to 80 organisms per cubic foot at a distance of 50 feet. Lesser numbers were produced by a primary sedimentation basin equipped with a surface spray, and none were recovered from an open digester or sludge bed.

The thesis by Albrecht shows that significant bacterial air contamination can occur through the agitation of sewage. The particles recovered were apparently quite large since the midjet impinger showed a recovery 31 times that of the Wells centrifuge. Such a result would be expected only if each particle contained many organisms. Large, multiple organism particles can be kept aloft in the high velocity outdoor winds while the residue forms an excellent protective coating. The larger particles thus assume a greater significance than in previous work concerned with indoor conditions and very low wind velocities.

The production of water droplets by collapsing cavities in a water surface was photographically investigated by Worthington and Cole in 1897 (6). With advances in high speed photography, it was possible in 1932 for Stuhlman (7) to show that bubbles burst upon reaching the surface of a liquid, thus leaving a cavity. The cavity rapidly collapsed under the influence of surface tension and gravity, and a jet arose from the bottom and extended above the surface of the liquid. Sufficient velocity was developed to extend the liquid jet to the point that surface tension caused disintegration into one or more droplets. These were projected as high as 14 centimeters into the air. Both height of ejection and number of particles varied with bubble size and both factors were maximized at air bubble diameters of 1.2 mm in distilled water and 1.5 mm in benzene. Benzene, with a lower surface tension, projected droplets only about one-half as high as did water. Stuhlman also observed that the process was greatly affected by the presence of surface films.

Both the jet-drop mechanism and the dependence of droplet size, number, and ejection velocity on the bubble diameter were verified photographically by Woodcock, et al. (8) in 1953 and by Kientzler, et al. in 1954. Droplet size was reported by Woodcock (3) to approximate one-tenth the bubble diameter for bubble sizes up to 2 mm diameter. However, with bubble diameters greater than 2 mm, all investigators found that the droplet diameter increased and ejection height decreased rapidly so that it might be difficult for droplets produced by 3 to 5 mm diameter bubbles to remain airborne. Although some 1 or 2 mm diameter bubbles are doubtlessly involved in sewage aeration, the majority might be expected to be somewhat larger. Therefore, the jet-drop mechanism might not produce a large number of droplets capable of remaining airborne.

Knelman, et al. (10), in 1954, found that in addition to the large drops produced by a bursting bubble, many smaller droplets were produced by the disruption of the bubble film upon bursting. Blanchard (11) and Newitt, et al. (12) stated that if such droplets were produced, they must be smaller than 1 μ diameter. Mason (13) reported that for bubbles 0.3 to 4.3 mm diameter, 100 to 200 droplets from 0.4 to 1 μ diameter were produced per bubble. Repeating the investigation on bubbles 0.25 to 2.15 mm diameter, Mason found 300 ± 80 droplets per bursting bubble.

Blanchard (2) found that the number of particles per burst was dependent upon bubble size and varied from about 5 or 10 for a 0.5 mm diameter bubble to 100 for a 4.2 mm diameter bubble. It was also reported that these droplets ranged in size up to 10 μ diameter and that their presence or absence was highly dependent upon the cleanliness of the liquid surface. Touching the finger to the liquid resulted in the complete

elimination of film droplets. However, if the bubble production rate was increased so as to form clusters on the surface, film droplet production was resumed. Facy (14) found large numbers of film droplets produced by bubbles bursting in clusters, but few from single bursts. Surface contamination may have accounted for the small number of droplets from single bubbles. Since sewage has an organically contaminated surface, film-drop production may depend upon the production of bubble clusters.

The mechanism of aerosol formation is important because of its influence upon particle sizes produced. In order that infection may occur from airborne pathogens, the particle must reach a vulnerable point in the respiratory or gastric tract. Hatch (15), Brown, et al. (16), and Hatch and Hemeon (17), among others, have shown that inert particles from 1 to 5 diameter can reach the alveolar section of the lung and are largely retained. Larger particles are retained in the upper respiratory tract and smaller particles are generally expired. Druett, et al. (18) found that although the 1-5 size range of Bacillus anthracis spores was most effective on a per organism basis in infecting guinea-pigs and monkeys, larger particles were capable of producing infection in the upper respiratory tract because of the overwhelming dose at the point of impact.

Aerosols arising from the ocean have been shown or deduced by several investigators. Boyce (19) found 5 to 200 diameter droplets produced by the bubbles from a breaking wave but none from the breaking action itself. Woodcock (20) concluded that eye irritation caused by the air in the vicinity of the "red tide" was a result of aerosolization by bubbles or an organic film on the surface of the water. Stevenson (21) found viable diatoms in the air above the surf zone in Texas. These were

of a characteristic and previously unknown type shown to be present in large number in the water.

Based upon the literature cited, it appears that some bacterial aerosols should be produced by bubbles bursting in sewage or polluted waters. Since Merz (4) found no coliform organisms downwind from an activated sludge aeration tank, it may be assumed that either very few aerosols were generated or the coliform organisms died rapidly in air. The large number of unidentified airborne microorganisms but few coliforms found by Albrecht (5) downwind from a trickling filter supports a conclusion that coliforms may be more sensitive to the airborne state than many sewage organisms unless they are absent from the surface layers.

The occurrence of various organisms in air has received a great deal of attention since Wells proposed the "droplet nucleii" mechanisms for transmission of respiratory disease (22). An extensive literature has developed, including several general reference works (23, 24, 25, 26). The most common topics include the factors affecting the death rate of airborne microorganisms, the relative resistence of various species, the distribution and control of potential pathogens in the indoor environment, and the use of microorganisms in biological warfare. Of primary interest to this study are those papers dealing with the survival of water and sewage bacteria and with the effects of constituents of the liquid from which the organisms are aerosolized.

Goodlow and Leonard (27) have presented an excellent summary of the factors affecting the survival of airborne bacteria. A partial listing of variables, along with additional selected references are listed below.

1. Specific species and strain of organism (28, 29, 30, 31).

2. Physiological state of the organism (32).

3. Organic and inorganic compounds present in the medium from which aerosolized (33, 34, 35).

4. Temperature and relative humidity of the air (28, 36, 37, 38, 39).

5. Sampling technique (40, 41, 42, 43, 44).

The inherent variability of experimental work involving bacteria in an adverse environment, along with a lack of standardized techniques has resulted in conflicting experimental results obtained by different investigators. Sampling techniques alone may introduce a difference of several hundred per cent in the number of viable organisms recovered. Kethley, et al. (42) found relative recoveries of some common samplers to be: 1.0 lpm critical oriface impinger - 100 per cent; single sieve sampler - 38 per cent; five sieve sampler in series - 52 per cent; Greenberg-Smith impinger - 50 per cent; midjet impinger - 42 per cent; 1.5 cfm nozzle in Greenberg-Smith tube - 35 per cent and 1.5 cfm nozzle in milk bottle - 75 per cent. Similar variations for some early sampling devices were reported by duBuy, et al. in 1945 (44).

Little work has been done using common waterborne pathogens. However, E. coli has been studied by many investigators and has been found to be typical of many water type organisms. Its resistance to the airborne environment is low, with 80 to 90 per cent loss in viability during the period of drying of an atomized droplet (33). Ferry, et al. (30) compared the resistance of Micrococcus candidus, Serratia marcescens, Escherichia coli, Mycobacterium phlei, and Corynebacterium xerose. Of these,

S. marcescens and E. coli showed only about 10 per cent remaining viability after initial drying, while the others ranged from 65 to 96 per cent viable. Wells and Stone (29) have generalized that water organisms are most sensitive, Streptococci are somewhat less sensitive, Staphylococci and Mycobacterium are resistant and that spores are very resistant to the aerosol environment. Therefore, while the coliform group may represent the behavior of pathogens in water, it probably does not typify the airborne behavior of many potentially pathogenic organisms that may be present in wastes.

The effect of the presence of chemical compounds upon the viability of bacterial aerosols has received a great deal of attention. Kethley, et al. (42) have studied the effect of chemical vapors as a means of disinfection, and also the influence on stability of the composition of the liquid from which aerosolized. Webb (33) studied the effect of many organic and inorganic chemicals added to the dispersion media in an effort to deduce the mechanism producing death. All inorganic compounds tested showed toxicity to E. coli and S. marcescens down to the limit of 0.1 molar concentration used. Toxicity was attributed to the chloride, sulfate, nitrate, and nitrite anions. Organic additives produced effects ranging from protection by a factor of 10 to almost immediate loss in viability.

Some additional factors affecting the aerosolization of bacteria by bursting bubbles include temperature, relative humidity, and ultra-violet radiation. In general, the loss in viability increases with higher temperature and decreases with higher relative humidity. High relative humidity, on the other hand, decreases the rate of evaporation

of aerosolized droplets, and might be expected to reduce the number of larger droplets which succeed in escaping the liquid surface to become airborne. Since it is well documented that certain frequencies of ultraviolet radiation are lethal to airborne microorganisms (42, 43, 44, 45), it may be assumed that optimum conditions for air pollution from water would occur on cool, damp nights.

Summary and Objectives

Although little information is available which is directly applicable to the topic under consideration, the related literature is sufficient to point out the large number of variables involved in any attempt to predict the number of viable organisms expected from a given sewage aeration unit or polluted watercourse. Some of the more important variables are:

1. Species of microorganism.
2. Physiological state of organism.
3. Concentration of microorganisms.
4. Sampling techniques, including apparatus and collection media.
5. Air and liquid temperatures.
6. Liquid pH.
7. Relative humidity.
8. Wind velocity.
9. Geometry of aerosol source.
10. Sunlight.
11. Surface contamination of liquid.

12. Nature and concentration of organic and inorganic compounds present in the liquid.

13. Size distribution of air bubbles.

14. Bubbles bursting in clusters or singly.

Since most of the variables listed would be expected to vary widely at a single treatment plant or stretch of river, it would be an impractical task to attempt to define the effect of each one. Moreover, the results could differ greatly between any two aerosol sources. With time and funds limited, it was felt that maximum information would result from a laboratory study where variance could be controlled within reasonable limits and the major factors influencing aerosol generation could be delineated. A wind tunnel was constructed which would allow control of the liquid medium, wind velocity, bubble size and generation rate, and of the microorganism itself. No attempt was made to control the temperature or relative humidity of the air, to evaluate the effects of geometry, or to consider the loss in viability beyond the initial evaporation of the droplet. The study was further restricted to the objectives listed below:

1. To determine whether viable bacterial aerosols can be generated by bubbles bursting in water.

2. To compare the survival of several species of the coliform group to that of other well-known species and to determine the effect of microorganism concentration.

3. To evaluate the effects of several additives on the generation rate and viability from water.

4. To determine the sizes of particles produced.

5. To estimate the importance of wind velocity, bubble rate, bubble size, physiological state and type of sampler used on the results obtained.

CHAPTER II

EXPERIMENTAL APPARATUS

Wind Tunnel

A wind tunnel was required which would provide control of wind velocity, relatively uniform velocity distribution over the cross section, easy access for rapid changes in experimental apparatus, exhausting of air outside the laboratory, and a bubbling chamber with a surface area sufficiently large to minimize bursting of bubbles at walls. The large volume of air required to operate the tunnel, along with decreased potential contamination made an outdoor intake preferable. These considerations, along with the unsuitability of alternate locations and the convenience of having the tunnel in the laboratory where bacteriological work would be performed, led to location in the Air Pollution Laboratory in the School of Civil Engineering. Since the laboratory was rather small and had windows on only two sides, a 120° bend was installed 4'-4" downstream from the sampling point.

Figure 1 shows the layout and dimensions of the floor of the tunnel. Features of importance include the sharp bend necessary to provide adequate lengths of inlet and sampling zones and relative locations of the bubble tank, the sampling points, and the point of velocity measurement. The height of the tunnel was 2'-5 1/2" throughout except for an abrupt enlargement to 3'-0" in the fan housing. Air movement was achieved by a

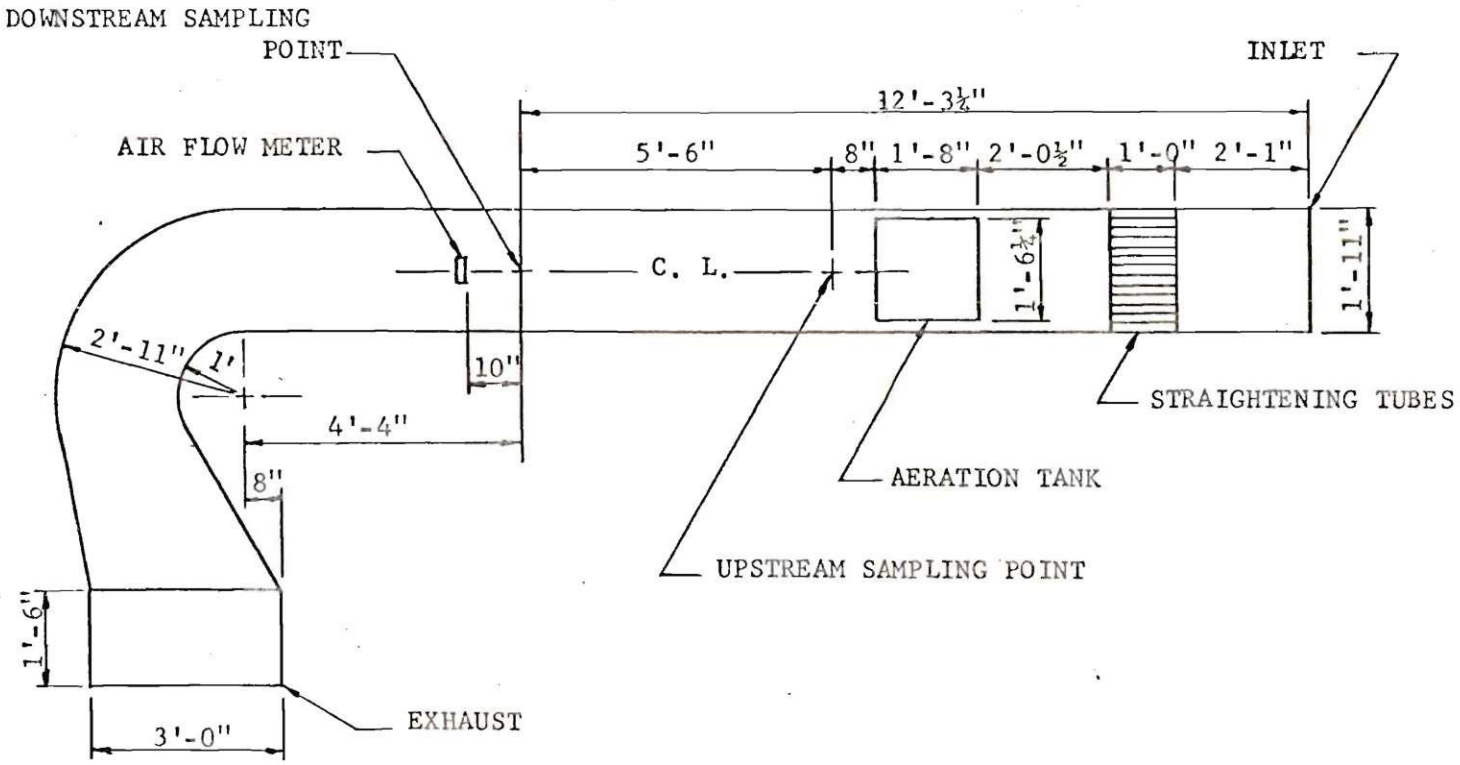

Figure 1. Floor Plan of Wind Tunnel

30" exhaust fan* powered by a 3/4 hp electric motor with a variable speed control unit. Power transmission was through a belt and pulley system with 10 to 1 reduction. Maximum fan speed was approximately 800 rpm with a corresponding wind velocity in the tunnel of about 10 fps. The fan was housed in a 3'-0" x 3'-0" x 1'-6" removable housing bolted to the tunnel.

Details of the tunnel may be seen from photographs of the side (Figure 2), bottom (Figure 3) and top (Figures 4 and 5). The entire top from the tank to the sampling point is hinged, gasketed with rubber tubing, and secured by five bolts with wing nuts. To provide a quick access to the wind velocity meter** for reading and resetting between samples, a plexiglass hinged port was provided over the sampling point. A second identical port was located in the top over the clear zone in front of the straightening tubes. The ports and side windows were of similar construction with wood-framed plexiglass set flush with the interior surface.

Locating the tunnel overhead in the laboratory was originally prompted by an effort to save space, but later proved advantageous to routine operation. With laboratory benches, ovens, hot plate, sink and hood located along the aisle below the tunnel, all operations except autoclaving and incubation were confined to an area approximately 8 feet wide by 24 feet long. Such an arrangement permitted the running of an experiment while performing other tasks such as cleaning glassware and counting colonies.

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*Dayton, "Hi-Volume" Industrial Exhaust Fan, 30", 10,900 cfm free air at 676 rpm.

**Taylor Biram's type anemometer, Fisher Scientific Co., Model No. 3132.

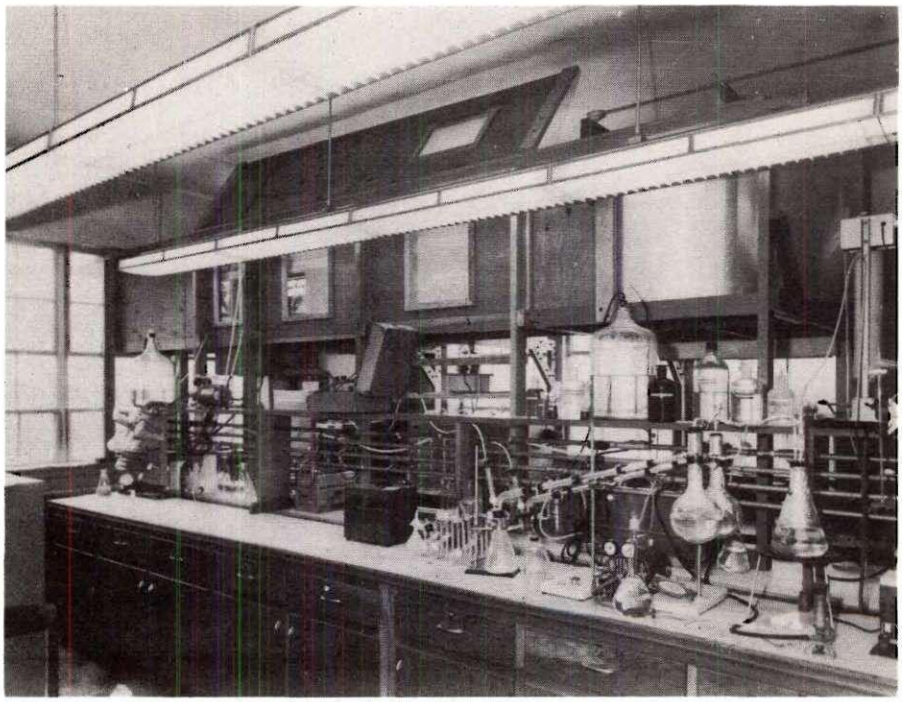

Figure 2. Side of Wind Tunnel

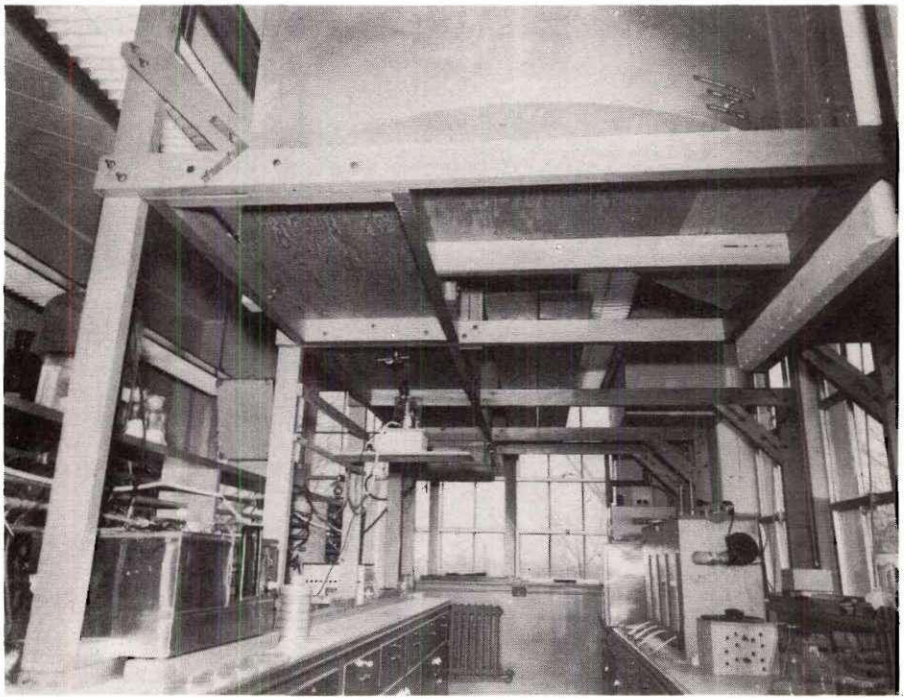

Figure 3. Bottom of Wind Tunnel Looking Upstream

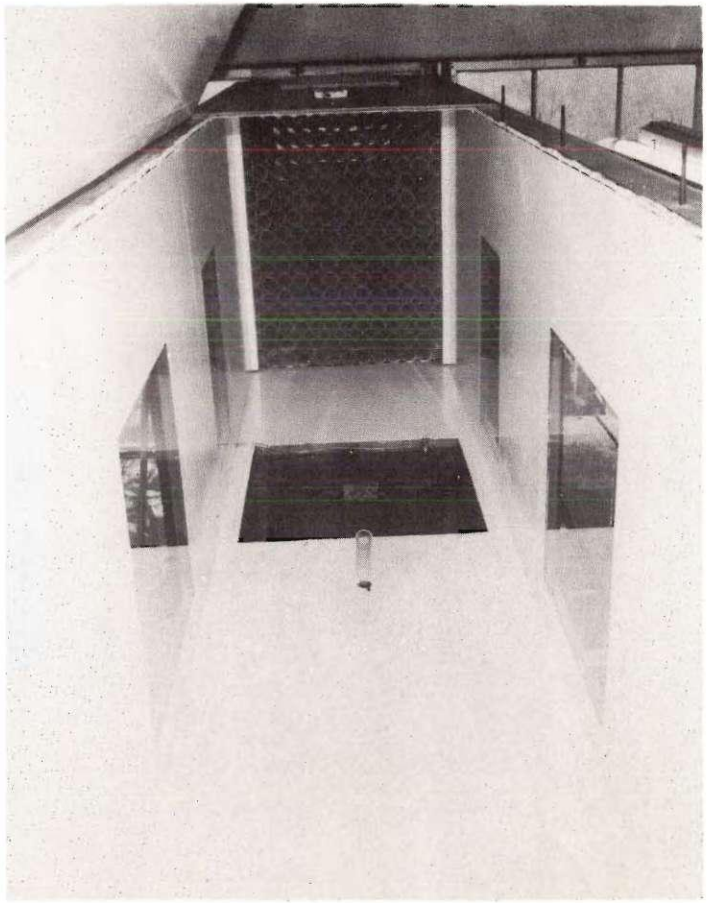

Figure 4. Interior of Wind Tunnel
Looking Upstream

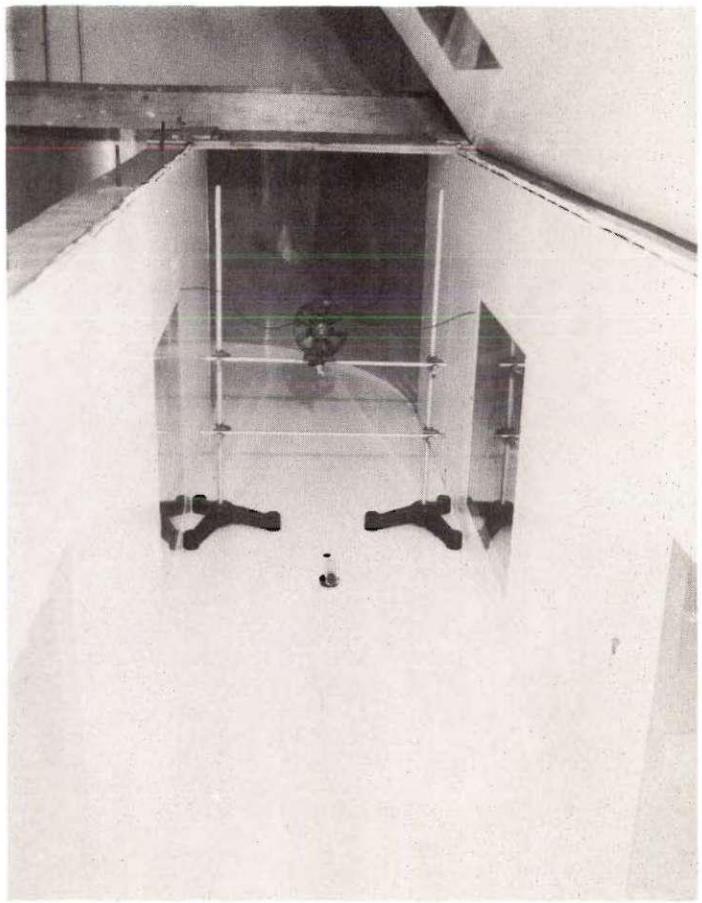

Figure 5. Interior of Wind Tunnel
Looking Downstream

The aeration tank was constructed of 3/4-inch plywood and lined with fiberglass. Inside dimensions were 1'-6 1/4" wide, by 1'-7 3/4" long, by 1'-0" deep. A filling and draining line, overflow, and sampling port were provided using glass tubing inserted through the bottom and connected to rubber tubing outside the tank. The overflow line proved to have inadequate capacity; so a simple inflow rate controller was fabricated by extending a piece of tubing from the tap to a point near the desired water level in the tank and back to waste. A branch led from the tubing to the tank inlet. By adjusting the inflow so that only a slight flow passed over the top of the loop in the tubing, an additional overflow route was obtained when the water level neared the maximum. The water level in the tank was maintained 3.7 cm below the upper edge through Experiment No. 32 and thereafter at 1.9 cm. Initial fluid volume was 69.0 liters and the later volume was 73.8 liters.

Several experiments were performed using settled and autoclaved sewage. Because of the difficulty in obtaining and autoclaving 20 gallon sewage samples, a miniature aeration tank was adopted. A jar of inside dimensions 4-1/4" wide, by 8-5/8" long, by 9-1/4" deep was placed in the large tank, shimmed flush with the tunnel floor; and a plywood panel was cut to cover the remainder of the regular aeration tank. It was not necessary to modify the bubbling system. Only 4.5 liters of aeration liquid were required.

Bubblers

Fritted glass bubblers* were used for most of the work reported.

*Will Corporation of Georgia, Atlanta, Georgia.

Initially, two bubblers of either coarse or medium porosity were located at third-points of the leading edge of the tank. Later it was found that a single bubbler was adequate for producing a uniform distribution of aerosols across the tunnel at the downstream sampling point. Air was supplied by a pressure pump* through a one-inch membrane filter, glass wool, a rotometer**, and 5/16-inch inside diameter "Tygon" tubing. A range of bubble sizes from 1 to about 5 mm diameter was produced by the medium bubblers with occasional bubbles up to 1 cm diameter being formed by the combination of several bubbles on the surface. A predominant size of 3-4 mm diameter was obtained. With the coarse bubblers, a predominant size of 4-5 mm was found with a lower limit of about 2 mm.

It was found that the fritted glass tended to clog with a bacterial growth after several experiments, resulting in increased head loss and decreased bubble size. Immersion of the bubblers in 50 per cent nitric acid between uses eliminated the problem.

A bubbler consisting of a horizontal piece of 1/2 inch pipe fitted with six equally-spaced hypodermic syringe adaptors to hold various size needles was tested as a means of obtaining a more uniform size of bubble. Although mean bubble diameter increased with needle diameter, a wide range in size similar to that of the fritted glass bubblers was obtained. As a result, very little work was performed with the hypodermic bubbler.

Microorganisms

Eight species of bacteria listed in Table 1 were selected for investigation. Since Merz (4) found no coliforms and Albrecht (5) found

*Will Corporation of Georgia, Atlanta, Georgia, Catalog No. 22691.
**Fisher Scientific Co., Catalog No. 11-164.

only a few arising from sewage treatment operations, while many aerobiologists have shown reasonable viability after short periods of time, it was considered of primary importance to determine how well several members of the coliform group could survive aerosolization from water and sewage. E. coli, A. aerogenes, E. freundii, and E. aurescens were chosen to represent the coliform group.

In order to determine the percentage of surviving organisms, it was necessary to determine the true generation rate of particles carrying bacteria. This determination was most readily accomplished through the use of spores of Bacillis subtilis var. niger since little or no loss in viability was expected from the evaporation of water droplets. A second reference organism was desired whose percentage survival would be less than that of B. subtilis but which would show useful recovery. A strain of Serratia marcescens was selected. This strain had been well characterized in prevous investigations at the Georgia Institute of Technology (32, 34, 35, 42, 49, 50).

Along with the coliform and reference organisms, it would have been most advantageous to study a group of pathogenic bacteria known to be present in sewage. However, since facilities for handling such organisms were inadequate and the wind tunnel was discharged to the outside air, it was necessary to use non-pathogenic species. Accordingly, S. durans from among the enterrococcus group and S. salivarius from the oral cavity were chosen.

Table 1. Identification of Bacteria Used

Species	Strain	Source
Escherichia coli	ATCC 10536	Dr. E. L. Fincher Engineering Experiment Station, Georgia Institute of Technology, Atlanta, Georgia
Escherichia freundii	ATCC 8090	ATCC*
Escherichia aurescens	ATCC 12814	ATCC
Aerobacter aerogenes	ATCC 129	ATCC
Streptococcus salivarius	ATCC 9222	ATCC
Streptococcus durans	ATCC 6056	ATCC
Serratia marcescens	ATCC 274	Prof. T. W. Kethley, Engineering Experiment Station, Georgia Institute of Technology, Atlanta, Georgia
Bacillus subtilis var. niger	Ft Detrick	US Army Chemical Corps Research and Development Laboratories, US Army Biological Laboratories, Ft Detrick, Frederick, Md.

*American Type Culture Collection, 2112 M Street, N.W., Washington 7, D.C.

Aerosol Samplers

Both the total number of viable particles produced and the size distribution of particles were of importance in this study. A slit sampler constructed at the Georgia Institute of Technology and patterned after a sampler described by Decker and Wilson (51) was chosen for the major portion of the work. Various forms of this device have been documented, but all have a narrow rectangular inlet through which air is drawn at high

velocity. Any particles contained in the air are impinged on the surface of agar in a container that may be removed for incubation. In order to distribute the particles so that they may be counted, either the slit or agar must be moved during sampling. Samplers of this type record the total number of particles containing viable organisms in the volume of air sampled.

The specific sampler used was composed of a base containing motor and gears, a revolving pedestal to hold a 20 mm x 150 mm Petri dish and a plexiglass cover containing the sample inlet and slit. The pedestal was adjustable vertically by means of an external knob; an indicator showed the correct position of the agar surface relative to the plane of the slit. The sampler was designed to operate at 1 cfm with 15 minutes being required for one revolution of the sample plate. Particles were deposited over a doughnut-shaped area on the agar surface. Since the aerosols from 15 cubic feet of air were deposited in a small area, the concentration of airborne particles was limited to 25-30 per cubic foot.

In order to estimate the size distribution of recovered particles, the Andersen (52) sampler was selected. This sampler, as does the slit, depends upon impaction of particles on an agar surface by abrupt change in direction of a high velocity jet of air. Air is drawn through 400 holes arranged in concentric circles in an aluminum plate. A special Petri dish is held beneath the holes to provide a collecting surface. Segregation of particles by size is achieved by arranging six basic units in series with each unit providing smaller-sized holes. Thus the jet velocity and the ability to remove smaller particles increases in each stage. Distance

between the plane of the jets and that of the agar surface is controlled by providing exactly 27 ml of agar in each of the specially molded Petri dishes. Air enters at the top of the sampler, passes through the first perforated plate, impinges on the agar surface, and travels around the Petri dish to enter the second stage. When operated at 1 cfm, Kethley (53) found effective diameters of particles trapped on each plate to be 9.8μ on the second stage, 6.2μ on the third, 3.8μ on the fourth, 2.2μ on the fifth, and 0.9μ on the sixth.

The low jet velocities in the upper two stages of the Andersen allow particles to be deposited over relatively large areas under each jet. Colony counts are therefore taken as the particle count. However, the higher velocities in the lower stages cause impaction directly at the point of jet impingement. Unless the number of particles collected per plate is less than thirty, error will arise from more than one particle passing through the same hole. The useful sample size may be increased significantly either by counting very young colonies in each deposit with the aid of a microscope (52) or by applying a statistical correction for overlapping to the number of positive holes out of a possible 400. A table for conversion of positive holes to most probable number of particles is provided by the manufacturer* with the sampler and is reproduced in abbreviated form as Appendix A.

In addition to the slit and Andersen samplers, six glass impingers for sampling bacterial aerosols** were obtained. These differed from

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*Andersen Samplers and Consulting Service, 1074 Ash Ave., Provo, Utah.
**Millipore Filter Corp., Bedford, Mass.

typical impingers in that they were provided with a long, gradually curved, 90° inlet, and an outlet for the aseptic removal of the impinger contents. Fifty ml of sampling fluid were required.

It has been shown to be desirable to provide isokinetic withdrawal of a sample from a moving stream of air (40). Therefore, a series of sample inlet tubes were fabricated* which consisted of a large diameter piece of glass tubing about three inches long, reduced in size, bent through 90° and joined to a verticle section of 6 mm inside diameter tubing. A sample inlet in place may be seen in Figure 5. Inlets having inside diameters of 18, 22, 25, and 31 mm were provided to allow the isokinetic withdrawal of 1 cfm of air at wind velocities of 6.1, 4.1, 3.2, and 2.1 fps, respectively.

The 6 mm straight tubing passed vertically through a rubber stopper fitted in a hole in the floor of the wind tunnel. The sample inlet tube was connected to the slit or Andersen sampler using an adapter improvised from the rubber portion of a Gooch crucible filter holder. Inlet tubes of either 6 inches or 1 foot 6 inches overall length were used depending on how far above the floor of the tunnel the sample was desired. The bend, constriction, and expansion in the inlet system probably discriminated against larger particles, but it was felt that the samplers would cause excessive obstruction of the air flow if placed inside the tunnel. Besides, the smaller particles, which should be relatively little affected by the inlet system, were considered of primary interest in the study.

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*Donald E. Lillie, Glassblower, Engineering Experiment Station, Georgia Institute of Technology, Atlanta, Georgia

Samplers were supported beneath the tunnel on a platform positioned at one of two levels. A sampler, with sample inlet, may be seen in position in Figure 3. By choosing the desired length of sample inlet tube, location of sampling platform, and shimming under the sampler, any sampling level from one inch to sixteen inches above the floor of the tunnel could be achieved.

Air flow through the slit and Andersen samplers was controlled by a 1.0 cfm critical oriface* and high capacity pump**. The exhaust from the pump was discharged to the laboratory air with no evidence of contamination.

*Gelman Instrument Co., Chelsea, Michigan, Catalog No. 7011.
**Wilson Products, Inc., Reading, Pa., No. 3A Impinger Unit.

CHAPTER III

EXPERIMENTAL TECHNIQUES

Preparation of Bacteriological Media

Commercial dehydrated media* were used in all bacteriological procedures with the exception of a modified tryptone glucose extract agar (42) employed in some early air samples for Serratia marcescens. Inorganic chemicals were of reagent grade.

Quantities of dehydrated broth or agar media were weighed into Erlenmeyer flasks and made up to the desired volume with demineralized water** of greater than 1 x 10^6 ohm resistance. The flasks were capped with aluminum foil and placed in a forced-draft drying oven at 150°C. After sufficient heating to insure solution, the media were transferred to screw-capped test tubes or aluminum foil capped Erlenmeyer flasks for immediate autoclaving. Broths containing sugars were autoclaved 10 minutes and other media 15 minutes at 121°C. After cooling, the containers were sealed as well as possible and were stored in the dark at room temperature until use. Broths were generally prepared once a week and agars twice.

M-Endo broth MF was used in the membrane filter technique for coliform analysis. Fresh solutions were prepared immediately before use by adding the required amount of water to a weighed quantity of dried media, adding two per cent of 95 per cent ethanol, and heating just to the

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*Difco Laboratories, Detroit, Michigan, or Baltimore Biological Laboratories, Baltimore, Maryland.

**Barnsted Still and Sterilizer Co., Boston, Mass., Mixed resin (red cap) in Bantam Demineralizer.

boiling point with frequent agitation.

Buffered dilution water as described in Standard Methods for the Analysis of Water and Waste Water (54) was prepared by adding 5 gms of K_2HPO_4 to 4 liters of demineralized water. The solution was dispensed in 99 ml portions into screw-capped Milk Dilution Bottles with the aid of an automatic dispensing burette*. The bottles were autoclaved 15 minutes at 121°C., cooled, and tightly capped until use. The residual volume after autoclaving was 95 ± 1 ml.

Bacterial Cultures

Pure cultures of vegetative organisms were carried in broth at 35°C. The coliform group was innoculated into lactose broth, the Streptococci into brain heart infusion, and Serratia marcescens into either lactose or nutrient broth. Both coliforms and Streptococci were transferred at 48-hour intervals into 10 ml of broth contained in a 20 mm x 100 mm screw-capped test tube. No evidence of contamination or deterioration of cultures was noted during the duration of experimental work. Serratia marcescens was transferred in a similar manner, but due to colony deterioration, the stock culture required differentiation by plating and selection of the desired strain about once a month. The growth of varients in this strain of S. marcescens has been previously documented by Kethley, et al. (49).

Cultures for experimental use were prepared by innoculation from a stock culture. Fifty ml or 100 ml of broth were measured into 125 ml or

*Will Corporation of Georgia, Atlanta, Georgia, Catalog No. 19841.

200 ml Erlenmeyer flasks. The flasks were capped with aluminum foil and autoclaved 10 minutes at 121°C. Reduction in liquid volume due to autoclaving was approximately five per cent. Prepared flasks could be stored in the dark at room temperature for 8-10 days without appreciable loss in volume or growth-supporting qualities. The culture media and length of incubation were varied, but typically were the same as those described for stock cultures.

Bacillus subtilis was obtained in the form of a dry powder with approximately 8×10^{11} spores per gram. Suspensions for experimental use were prepared by weighing a quantity of spores into a milk dilution bottle and adding the desired amount of sterile buffered dilution water.

Preparation of Aeration Liquid

Several coatings of fiberglass resin were applied to the inside of the tank after construction before all leaks were stopped. The resulting rough bottom surface did not permit complete drainage of aeration liquid. In order to prevent significant carry-over of microorganisms, it was necessary to rinse the tank in tap water before and after each experiment. Therefore, the first step in aeration tank preparation was to fill the tank from 1/3 to 1/2 full of water, to scrub the sides and walls, to drain, and to rinse with several liters of clean water. Using this simple procedure, carry-over of organisms was insignificant. It should be noted that no attempt was made to maintain a pure culture of the test species in the aeration tank. Background organisms from air and water were found to be at a level of about one per cubic foot sampled. These extraneous

growths were easily distinguished from the test colonies and appeared to have no adverse effects on results.

After cleaning the tank, the inlet line was connected to the discharge line from the demineralizer or directly to the tap. The tank was filled and allowed to overflow at least 15 minutes to provide a clean water surface. While the tank was overflowing, the sampling tube was rinsed by wasting some water through it. The inlet was then clamped off and the water was allowed to equilibrate at the overflow level. Chemicals were added and the tank contents were thoroughly mixed with a large glass stirring rod. The desired volume of liquid culture was then stirred into the liquid. The tunnel was quickly sealed, the exhaust fan and air bubbler pumps were cut on, and flows were adjusted to the desired values. After a period of about 15 minutes to allow further mixing and stabilization of the surface bubble pattern, the first air sample was taken.

Average chemical characteristics of Atlanta tap water are shown as Appendix H. It was found necessary to add 7 mg/l (0.52 gm) of sodium thiosulfate to insure complete removal of residual chlorine.

Concentration of Bacteria in the Bubble Tank

Samples of 5 to 10 ml were withdrawn from the tank through the sampling tube into sterile 10 or 25 ml Erlenmeyer flasks. Approximately 25 ml of liquid were wasted to flush the tube before sample collection. A portion of the sample was then transferred through the desired dilution sequence using sterile volumetric pipettes and the milk dilution bottles previously described. Dilution bottles were shaken violently for at least 30 seconds to provide uniform dispersion of microorganisms.

The membrane filter technique (54) was used in all coliform determinations except those where a non-coliform organism was simultaneously employed. Apparatus consisted of 47 mm diameter filters, 50 mm diameter plastic Petri dishes, and glass filter holders, all of which were produced by the Millipore Filter Corporation*. Membrane filter holders were autoclaved 10 minutes at 121°C. before use. Petri dishes were reused after carefully washing, soaking a minimum of 24 hours in 70 per cent ethanol and drying inverted on clean paper under an ultraviolet lamp in a hood without air circulation.

All manipulations in the membrane filter analysis were performed in the hood after reducing possible contamination with at least one hour of ultraviolet radiation. Petri dishes were prepared immediately prior to an experiment by placing an absorbent pad in the dish and adding 2.0 ml of M-Endo broth MF. Filters were removed from the envelope with alcohol-flamed tweezers and placed in the filter holder. A suitable aliquot from 5 ml to the entire contents (105 ml maximum) of the diluted water sample was filtered under mechanical vacuum. The walls of the holder were then rinsed with sterile dilution water, the filter was carefully placed in a Petri dish, and the dish was inverted and incubated. Triplicate portions of each sample were filtered. Incubation at 35°C., for 15 to 18 hours produced maximum counts of uniform colonies with full sheen development. Counting was performed under a 10-power, dissecting, binocular microscope with the aid of a hand tally counter. Up to 200 colonies per filter could be reliably counted in this manner.

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*Millipore Filter Corp., Bedford, Mass.

Plate counting was performed in a normal manner. Three one ml portions of a diluted sample were transferred into 100 mm glass Petri dishes. Approximately 10 ml of agar were added, the plates were swirled gently and allowed to solidify before inverting in the incubator. The agar was held at 45-48°C. in a water bath during the experiment. Counting, with the aid of a Quebec colony counter, was accomplished after 15-18 hours incubation at 35°C. except for the Streptococci which were incubated 48 hours. An attempt was made to obtain 100 colonies per plate to achieve reliability of results along with ease of counting.

Air Sampling

Air samples were collected using the impinger, slit and Andersen samplers previously described. Since techniques of preparation and collection differed for each, it will be necessary to present these separately.

The impingers required a relatively large amount of manipulation in preparation and analysis. The two halves of the impinger were wrapped in kraft paper and autoclaved after capping the inlet tube, the suction outlet, and the sample withdrawal tube with aluminum foil. A short piece of "Tygon" tubing was required as an extension of the sample withdrawal tube to allow it to be clamped off. This was prepared separately by immersion in 70 per cent ethanol for a minimum of 24 hours. Also required in sterile condition were the sampling fluid, Dow-Corning Antifoam B*, a glass rod, a 50 ml graduated cylinder, small flasks of dilution water, and

*Dow Corning Corporation, Midland, Michigan.

empty dilution bottles graduated at 99 ml.

In order to minimize contamination, all impingers required for an experiment were prepared at one time after ultraviolet irradiation of all necessary apparatus for one hour. The preparation procedure involved unwrapping and assembling the two parts of the impinger, whose sterility was then maintained by the aluminum foil caps placed over all openings before autoclaving. A section of "Tygon" tubing was removed from the alcohol bath and quickly pushed onto the sample outlet tube. The aluminum foil cap removed from the sample outlet was then pinched over the exposed end of the tubing and the tubing was clamped. Fifty milliliters of sampling fluid were measured into a graduated cylinder and quickly poured into the impinger. The impinger tube was then lifted and a drop of Antifoam B was placed near the tip of the impinger with the aid of a glass rod. The prepared impinger was then clamped in a vertical position until use.

Samples were collected at either of two positions in the tunnel. In the upper position, the impinger was clamped to a laboratory stand inside the tunnel. Due to the length of the impinger, the sample inlet could not be less than 17 inches above the tunnel floor. In the alternate position, the inlet was inserted into the tunnel through a hole in the floor. The inlet tube was positioned about three inches above the floor. Impingers were operated at critical velocity, which yielded a sampling rate between 9 and 10 lpm, depending upon the particular impinger used.

After a sample had been collected, the fluid was withdrawn, under the hood, into a sterile dilution bottle. Approximately 45 ml of the original 50 ml volume were recovered. At least 3 rinses of the impinger

were employed to insure the maximum removal of cells. The sampling fluid and rinse water were made up to an estimated 100 ml in a dilution bottle calibrated at 99 ml. Suitable aliquots were then transferred into 95 ml portions of buffered dilution water and were filtered through membrane filters, placed on nutrient media, incubated, and then counted.

The slit sampler required less manipulation than either of the other samplers. It was found unnecessary to sterilize the sampler between or during experiments. Consequently, only the preparation and incubation of a single Petri dish per sample were required. Approximately 30 ml of agar at 45°C. were poured into 15 mm x 100 mm plates resting on a level surface. After hardening, the plates were inverted and stored at room temperature until use. Plates were prepared the afternoon of the day preceding the experiment. It was found that "Kimax"* plates performed better than "Pyrex"** because of their greater weight and level bottom surface.

A plate was inserted into the sampler, adjusted into position, and the sampler was positioned and connected to the inlet tube. After drawing air at 1.0 cfm through the sampler for the desired length of time, the plate was removed, allowed to warm to room temperature and incubated for 15-18 hours (approximately 48 hours for Streptococci) at 35°C. Colonies were counted with the aid of a Quebec colony counter.

The Andersen sampler required the preparation of large numbers of Petri dishes, since six were required per sample. In addition, exactly 27 ml of agar were required per dish. An attempt was made to measure

*Kimble Glass Co., Toledo, Ohio.
**Corning Glass Works, Corning, New York.

volumes of agar into large test tubes that would produce 27 ml after autoclaving, but the variation in volume was too great. Therefore, sterile agar was measured from a 250 ml dispensing burette, capped with cotton inside of an aluminum foil cover.

Agar was dispensed at a temperature of 45 to 50°C. to prevent excessive evaporation. The low temperature required that dishes be filled rapidly since the agar would solidify in the burette tip if left motionless longer than 30 seconds. Sufficient continuity was achieved with the aid of plywood strips holding 4-6 dishes each. The dishes were poured, put aside to cool as a group, and a new set of dishes on a plank put into position. In this manner, dishes could be poured at a rate of 150 per hour without clogging the burette tip. Plates were prepared in the afternoon of the day preceding the experiment and were stored at room temperature until use. Incubation of typical prepared plates very rarely showed contaminating organisms.

As with the slit sampler, it was found unnecessary to sterilize the Andersen between samples. The prepared plates were inserted, the sampler positioned and connected to the sample inlet, and air was drawn through under vacuum at 1.0 cfm. The plates were removed, allowed to warm to room temperature (about 15 minutes) and were incubated and counted as previously described.

Experimental Procedures

The more important procedures required for an experiment have been discussed above. In order to relate these and to integrate minor operations, previously unmentioned, an outline of a typical experiment will be presented.

An experiment normally required four days in sequence. On the first day, duplicate flasks of media were innoculated with the desired culture and were incubated. On the second day, media were prepared and dispensed and the necessary glassware cleaned and sterilized. The third day was devoted to the experiment itself and the fourth to counting colonies and samples and to cleaning up. Obviously, overlap of experiments was quite practical and an average of about three per week was achieved.

The first task in performing an experiment was to rinse and fill the aeration tank. While the tank filled, dilution bottles and Petri dishes were set out and numbered, chemicals were weighed onto aluminum foil strips, and the samplers and inlets were prepared and put into place. After the tank was full, chemicals and culture were added, the air bubbler pump and tunnel fan were started, and the top of the tunnel was sealed. While the aeration tank stabilized, the air temperature and relative humidity were measured outside the window at the mouth of the tunnel, and where necessary, the pH of the tank liquid was measured. Prior to sample collection, the bubbler air flow and the wind velocity were readjusted to the desired values.

To collect a sample, the sampler was first lifted into position and fitted to the sample inlet. The inlet was turned to point upstream and the air meter zeroed through the access port over the sampling point. Then, from a position under the tunnel, the air meter was cut on, a stop watch started, and the sampling pump started. While the sampler was running, the first bacteriological water sample was withdrawn from the aeration tank, diluted and plated. At the end of the required sampling

period, the air meter and sampling pump were stopped, and the sample plate was quickly removed and replaced with a sterile plate. After reading and resetting the tunnel air meter, the sampler was once again put into position for a second sample. From two to three minutes were required between samples. Data sheets used for experiments employing the slit and Andersen samplers are shown as Appendix B.

CHAPTER IV

RESULTS

General

Glass impingers were used almost exclusively for sampling during the first sixteen experiments. All data were erratic and a tendency of contaminating organisms and S. marcescens to spread made colony counting very difficult. As a result, the use of the impinger was discontinued and all data have been omitted from the present account. A number of later experiments were also omitted due to errors in experimental techniques.

In order to conserve space, the bulk of the data have been divided into two sections. Appendix C contains the results of experiments in which the slit sampler was used and Appendix D contains the results of sampling with the Andersen. Data are listed in each case under the original experiment number and in sequence. Where both the slit and Andersen samplers were employed in a single experiment, the experiment number appears in both Appendices. Several experiments which were not adaptable to the form of Appendices C and D are included in special Appendices. The discussion of results following in this chapter is independent of the actual time sequence of experimentation.

Bacterial Assay

In order to estimate the precision of the pour plate and membrane filter techniques for enumeration of bacteria in the liquid aeration medium, the data were expressed in terms of organisms per milliliter

recovered per milliliter (or milligram) of culture added to the tank. The results are shown in Table 2. The standard deviation of the population was estimated, using quality control techniques (55), from several small sample groups. If measurement techniques alone affected the apparent yield, predicted concentrations using means of three samples could be expected to show a coefficient of variation of 0.056 for B. subtilis on pour plates.

The actual yields and standard deviations for the organisms used are shown in Table 3. As expected, the coefficients of variation are much greater than those predicted by variations in measurement technique alone. The greater dispersion reflects the variations resulting from weighing or measuring the culture, growth rate, nutrient concentration in culture media, etc.

The coefficient of variation in experiments for which S. marcescens was determined using membrane filters appeared quite large when compared to that obtained with pour plates, while the mean yield was smaller. This trend was observed during the course of the work and was checked by performing duplicate membrane and pour determinations for two experiments. One of these produced comparable yields while the other resulted in a 100 per cent greater yield using pour plates. Since, C_V for the two techniques did not show a significant difference, it appears that the response on membrane filters varied from day to day. Similar results have been obtained by others (56, 57, 58). Therefore, pour plates were used exclusively for S. marcescens in later experiments.

The other organisms with the exception of S. salivarius showed reasonable variations in yield. Differences in S. salivarius culture

mass were observable under apparently identical conditions, however, so the dispersion was attributed to natural variation in growth.

Table 2. Evaluation of Membrane and Pour Plate Techniques

Organism	Technique	No. Samples	Mean Yield per ml (mg) Added	σ	C_V
B. subtilis	pour	25	0.86×10^4	0.083	0.097
S. marcescens	pour	16	0.68×10^4	0.098	0.14
S. marcescens	membrane	23	0.46×10^4	0.078	0.17

Air Sampling

In order to determine the statistical validity of measured differences in aerosol concentration, it was first necessary to estimate the standard deviation of air samples. In several sample series, it was noted that neither the standard deviation nor the coefficient of variation was a numerical constant, but varied with the number of organisms collected. Therefore, it appeared advisable to determine an expression for the relationship between some measure of variance and the number of organisms sampled, based on a large number of small sample groups.

Table 3. Yields of Microorganisms in Culture

Organisms	Technique	No. Experiments	Mean Yield Per* ml(mg) Added	σ	C_V
B. subtilis	pour	23	0.91×10^4	0.098	0.11
S. marcescens	pour	10	0.65×10^4	0.066	0.10
S. marcescens	membrane	15	0.45×10^4	0.21	0.47
S. salivarius	pour	8	0.44×10^4	0.24	0.55
E. coli	membrane	4	0.30×10^4	0.089	0.30
E. aurescens	membrane	5	0.37×10^4	0.087	0.24
S. durans	pour	5	0.54×10^4	0.20	0.37
E. freundii	membrane	3	0.35×10^4	0.13	0.37
A. aerogenes	pour	7	0.74×10^4	0.19	0.26
A. aerogenes	membrane	5	0.77×10^4	0.21	0.27

A plot of the log of the coefficient of variation as a function of the log of the number of samples collected is shown as Figure 6. The data, shown in Appendix E, represents twenty-nine groups of from four to twelve slit samples and several species of microorganisms. The dispersion is great, but is expected, due to the small size of sample groups. A straight line was fitted by the method of least squares, which resulted in the equation:

$$C_V\ (\%) = \frac{85.4}{N_s^{0.434}} \qquad (1)$$

*Multiply by 6.38×10^4 to convert to no. of organisms per ml culture. B. subtilis only is expressed in mg and should be multiplied by 6.38×10^7 to obtain organisms per gram dry spores.

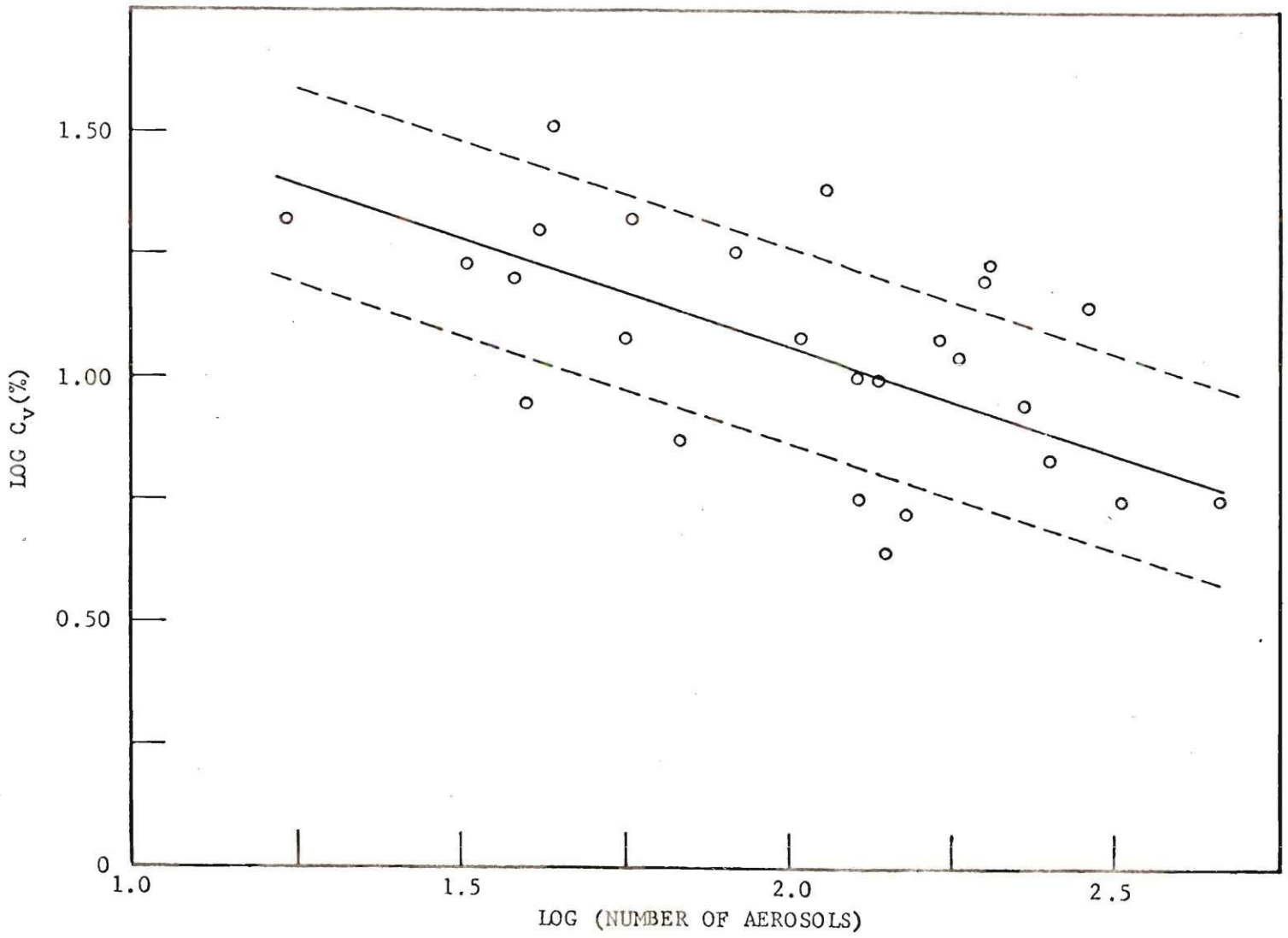


Figure 6. Coeficient of Variation of Air Samples

This relationship along with 95 per cent confidence limits, is plotted in Figure 6.

Using Equation 1, the significance of the difference in means of two sample groups may be established (55). The difference in means must exceed that difference which may occur due to chance alone at a given level of probability.

$$\bar{x} - \bar{y} \geq t_{\alpha}\,;\; n_x + n_y - 2 \sqrt{\frac{\sigma_x^2}{n_x} + \frac{\sigma_y^2}{n_y}}$$

The left hand side may be computed using the number of samples (n), the sample means ($\bar{x}$ and $\bar{y}$) and the standard deviation (σ) computed from Equation 1 ($\sigma = C_v \bar{x}$). For a significant difference, the value must be exceeded of the t-distribution for $n_x + n_y - 2$ degrees of freedom and selected probability due to chance occurrence.

The slit and Andersen samplers are similar in principle of operation and the variance of samples was suspected to be largely due to random fluctuations in aerosol concentration. Therefore, it was considered unnecessary to develop a separate relationship for the coefficient of variation for the Andersen sampler. The relative recoveries of the two samplers were compared, however, for ten experiments in which both types of samplers were used. The number of organisms collected by the slit sampler divided by the number collected by the Andersen was found to average 1.18 and to differ significantly from 1.00 ($p = 0.025$). The data and computations are shown in Appendix F.

Distribution of Aerosols in Tunnel

In order to relate the concentration of aerosols found at the normal downstream sampling point with the rate of generation, samples were collected

at five points across the tunnel and at four or five positions vertically. The data are shown in Appendix G. It was found that short circuiting due to the bend in the tunnel produced a large difference in concentration across the section. However, the distribution was sufficiently close to linear, that samples could be averaged across the tunnel to produce a single concentration corresponding to each elevation.

The vertical distribution was related to the downstream sampling point at the center line and 2.25 inches above the floor by dividing the number per cubic foot at each elevation by the number per cubic foot found at the reference point. A log-log plot of relative concentration as a function of height is shown in Figure 7. A least squares fit of a straight line through the data produced the equation:

$$\frac{C}{C_o} = 2.25\ H^{-0.922} \qquad (2)$$

where C = No./ft^3 at elevation H.
C_o = No./ft^3 at C.L. and H = 2.25 inches.
H = Height above floor in inches.

It was impossible to determine the validity of the equation at small values of H. However, the greater turbulence near the floor would tend to cause equalization of concentration. Therefore the relative concentration was considered to be constant at a value of 1.19 from 0 to 2 inches and to be represented by the equation between 2 and 30 inches.

The number of aerosols generated per second may be expressed as:

$$R = C_{ave.}\ AV$$

where R = No. aerosols/sec.
$C_{ave.}$ = Average No. aerosols/ft^3 across the tunnel.
A = Cross sectional area of tunnel-ft^2.
V = Air velocity - ft/sec.

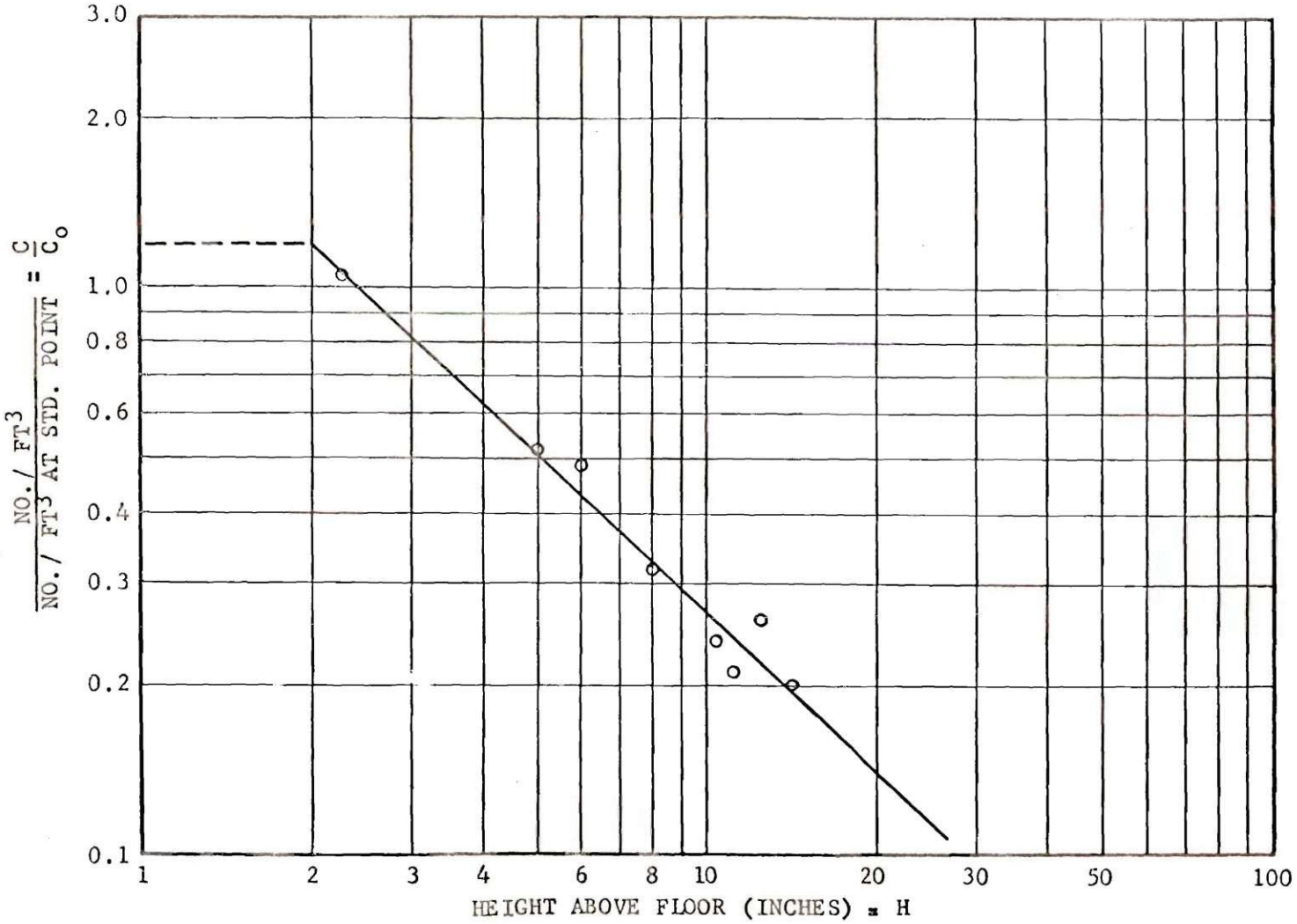


Figure 7. Vertical Distribution of Aerosols at Downstream Point

Considering vertical distribution as outlined above:

$$\left(\frac{C}{C_o}\right)_{ave.} = \frac{1}{30}\left[\int_z^{30} \frac{C}{C_o}\, dH + 2 \times 1.19\right]$$

$$= \frac{1}{30}\left[\int_z^{30} 2.25\, H^{-0.922} dH + 2.38\right]$$

which may be integrated to obtain:

$$\left(\frac{C}{C_o}\right)_{ave.} = 0.332$$

or since C_o is assumed constant:

$$C_{ave.} = 0.332\, C_o$$

Substituting $0.332\, C_o$ for $C_{ave.}$, 4.70 ft^2 as the area of the tunnel, and 4.1 ft/sec as the desired velocity (isokinetic sampling velocity of standard intake) yields:

$$R = 6.4\, C_o \qquad (3)$$

Equation 3 was used to convert the concentration measured at the single sampling point to the number of aerosols per second passing the section.

Effect of Wind Velocity

It was found that velocity in the tunnel fluctuated under the influence of outdoor winds. As a result, isokinetic sampling velocities could not normally be maintained. The variation also prevented direct comparison of aerosol concentrations at higher and lower velocities with that at the desired velocity. An approximate measure of the influence of velocity on concentration was obtained from Experiments 22, 23, 100 and

119, which were composed of samples collected at various wind velocities. Each sample concentration was divided by the mean concentration for its experiment, then was multiplied by the measured wind velocity and was plotted as a function of velocity. The resultant distribution is shown in Figure 8. A straight line fitted by the method of least squares produced the equation:

$$\frac{C}{C_{ave.}} V = 1.75 + 0.662V$$

which when adjusted to correspond to the conditions $\frac{C}{C_{ave.}} = 1.00$ at V = 4.1 fps, yielded:

$$\frac{C}{C_{4.1}} = \frac{1.39}{V} + 0.662 \qquad (4)$$

an arithmetic plot of which is shown as Figure 9. Equation 4 was used to correct data to its equivalent at 4.1 fps.

Effect of Bubble Size and Flow Rate

No attempt was made to accurately define the influence of bubble size or air flow rate on the aerosolization of bacteria, since the bubble generation system used has no counterpart in nature or in a sewage treatment plant. The system was designed only to produce a range of bubble sizes that might be expected to be present and that would be expected to produce aerosols. The standard air flow rate of 0.5 lpm was selected to produce a large number of bubbles while minimizing bubble mergers.

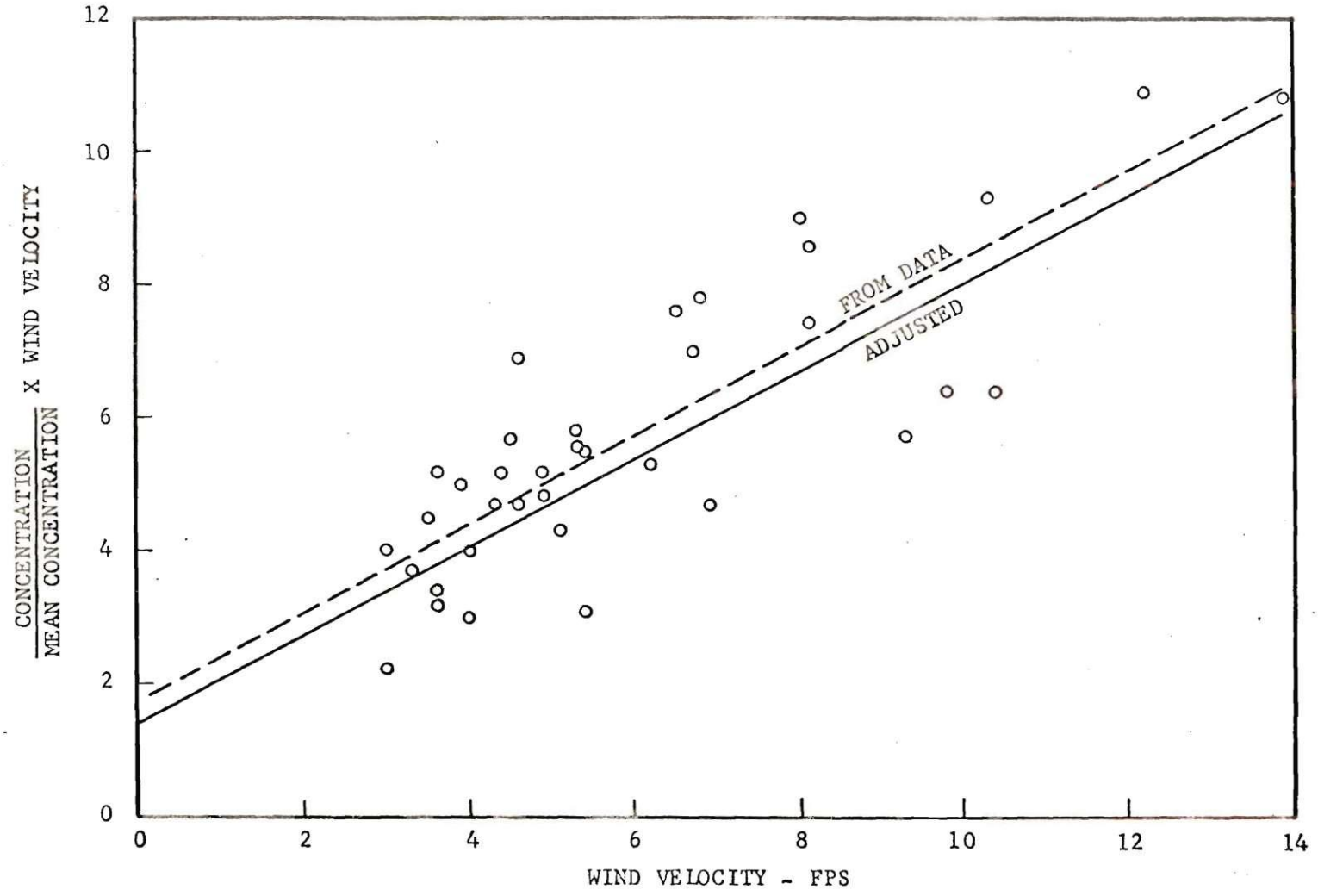


Figure 8. Effect of Wind Velocity on Aerosol Concentration

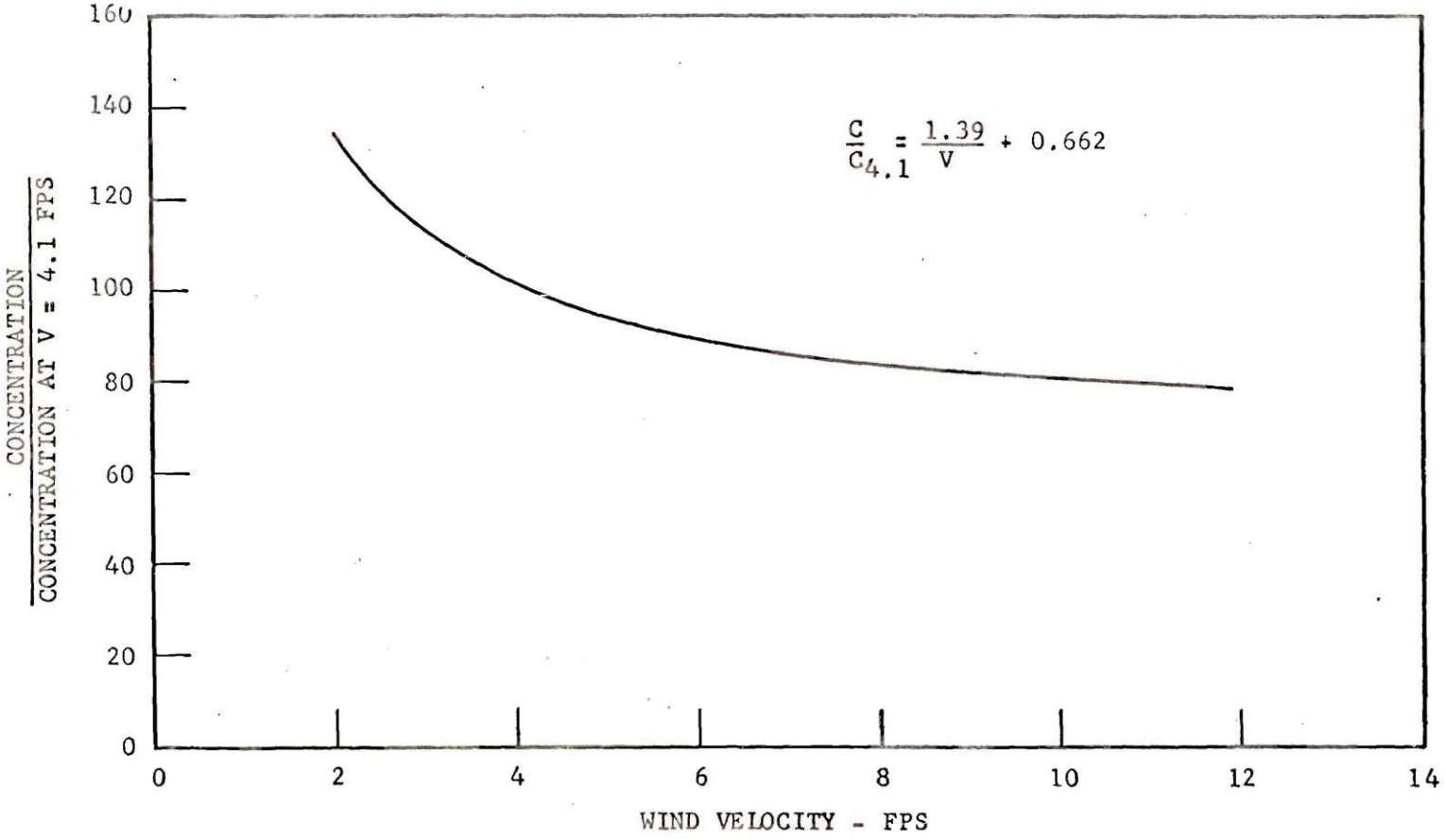


Figure 9. Variation in Aerosol Concentration with Wind Velocity

As a guide to the effect of air flow rate on the bubbling system employed, the results of two experiments are shown in Figure 10. Using two coarse bubblers, the aerosol production was found to be proportional to the air flow rate. A single, medium bubbler, however, showed the logarithm of the generation rate to be proportional to the air flow. If the distinction is real, it may be due to the difference in physical behavior of the aeration liquid employed. The coarse bubbler was used with demineralized water with peptone and potassium phosphate added, while the medium bubbler was used with tap water. Demineralized water produced bubble clusters at all air flow rates, while clusters occurred in tap water only at higher flow rates.

Neither number of aerosols generated nor size distribution were conclusively shown to be dependent upon bubble diameter. With the aeration systems used, including coarse and medium bubblers and several sizes of hypodermic needles, a range of bubble sizes was produced and only the mean diameter was varied. It was expected that the number of particles generated would decrease with increasing bubble diameter, but if the production of droplets depends upon the presence of bubble clusters, this may not be true.

Extended Aeration

Kethley, et al. (32) have shown that the ability of S. marcescens to survive in air depends upon the physiological state of the culture. During periods of adjustment to a new environment or while actively multiplying, the viability is lower than that of a static culture. This effect, in several relatively long experiments, is shown in Figure 11.

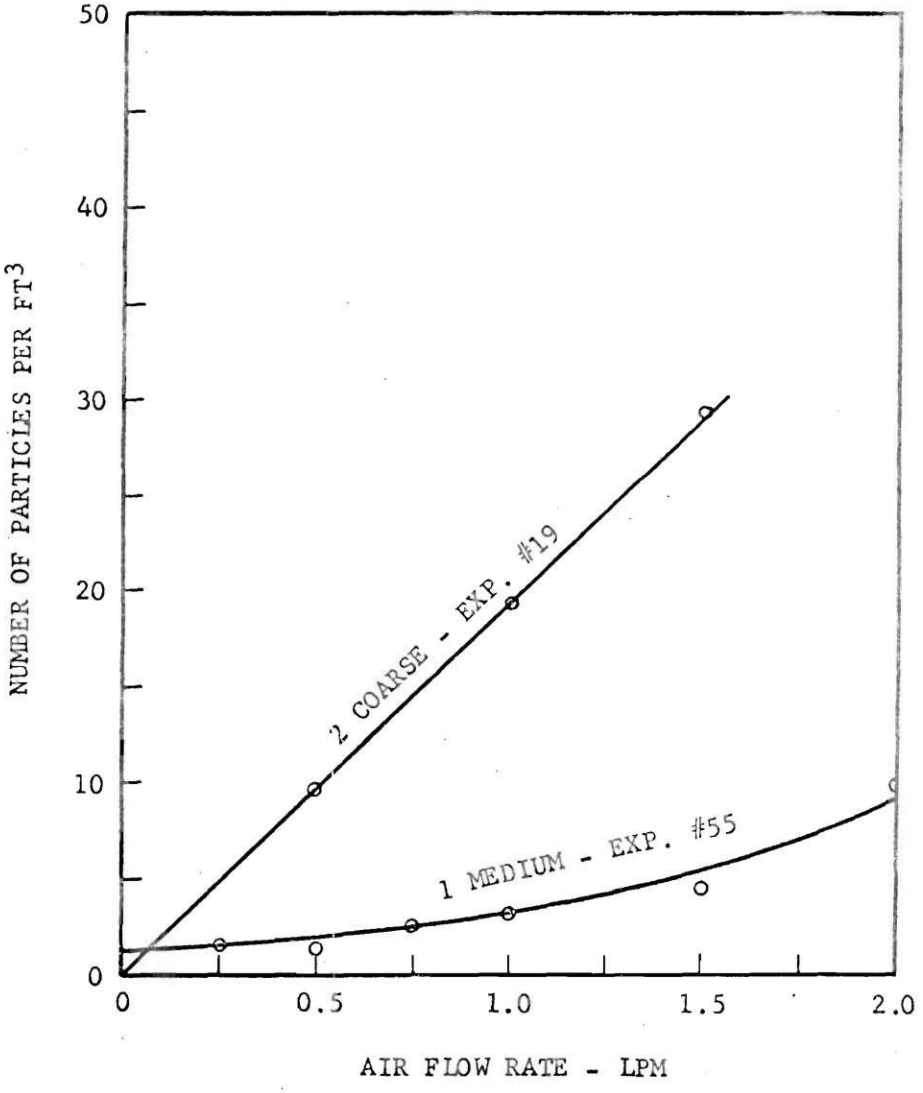


Figure 10. Effect of Air Flow Rate on Aerosol Production

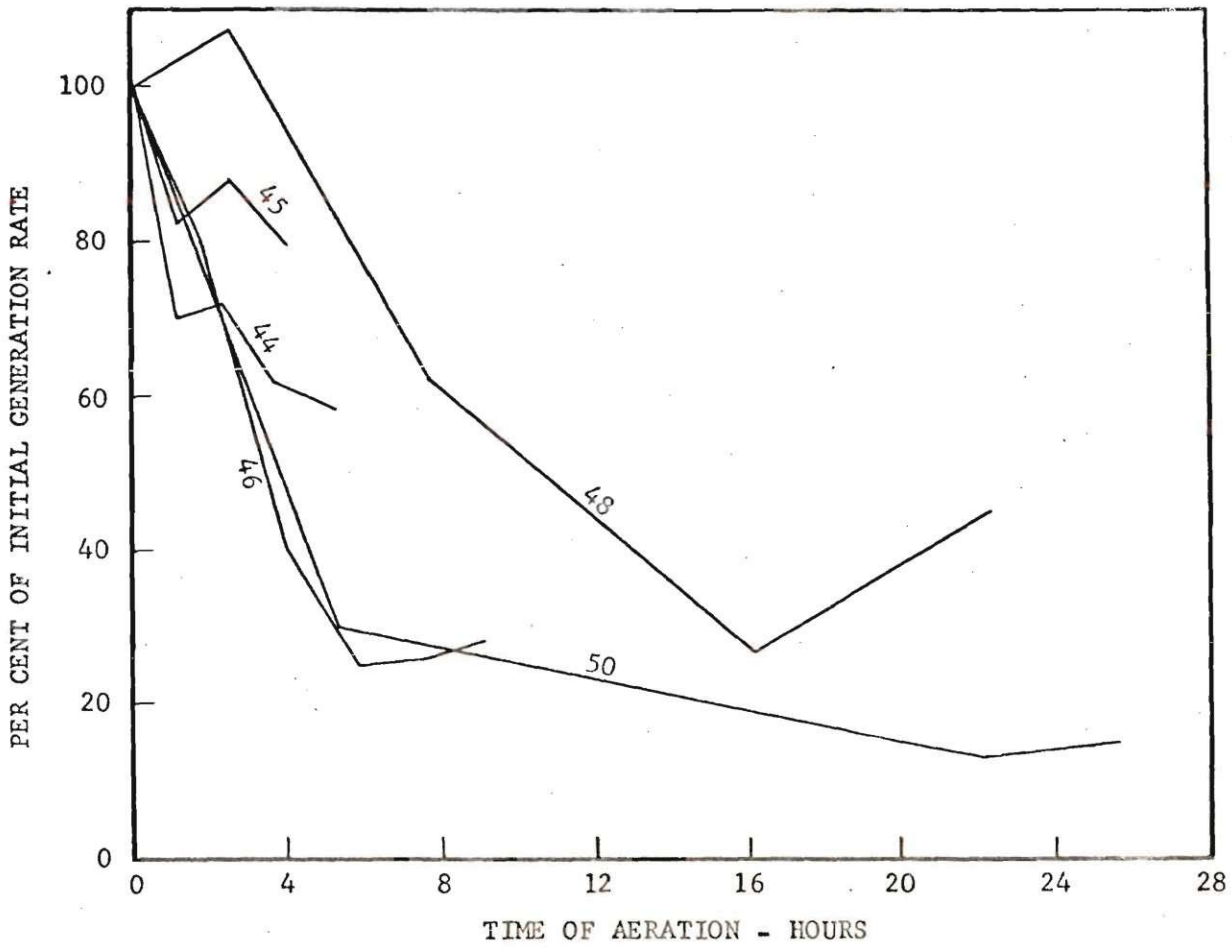


Figure 11. Effect of Aeration Time on Recovery of S. marcescens

The number of bacteria per milliliter of water remained constant or decreased slightly in all experiments except No. 50 where the last two samples showed an increase. The sharp initial drop was not noted in all S. marcescens experiments of the normal 2 to 4 hours duration, nor with other species with the possible exception of S. durans. However, B. subtilis showed a tendency to produce a slightly lower value for the initial group of samples, and to remain constant thereafter.

Where possible, as in upstream versus downstream aerosol concentrations, samples were alternated so as to eliminate the effect of any change in potential viability. If alternation of samples between the desired conditions was not possible, the experiment was performed as rapidly as possible. Only one sample could be collected at a time and typically required 20 minutes. In order to hold the duration of an experiment to 3 to 4 hours, it was necessary to employ means of only 3 to 5 samples. Although sample groups of 3 are unusually small, a difference in means of approximately 20 per cent was typically significant at the 5 per cent level and was considered adequate for the present endeavor.

Recovery of Coliform Organisms

A number of attempts were made to recover organisms of the coliform group at the downstream sampling point. Although culture age, concentration of organisms, and type of aeration liquid were varied, only E. aurescens was found in small, but significant, concentrations. A summary showing experimental conditions is shown in Table 4.

Table 4. Summary of Coliform Recovery Experiments at Downstream Point*

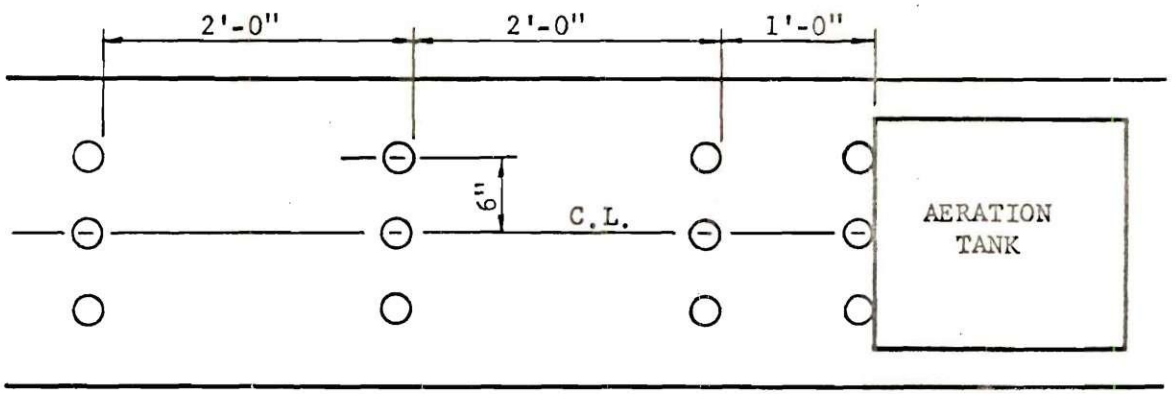
Exp. No.	Species	Liquid	No./ml x10^{-5}	Age of Culture (hrs)	Type Agar	Air Temp. °F	Rel. Humid. (%)	Colonies per 15 ft^3	Background** Colonies per 15 ft^3
17	E. coli	DKP	2.3	73	EA	-	-	0	-
41	"	DKP	2.6	46	BHI	50	58	0	0
53	"	TP	1.8	44	BHI	25	-	9	7
60	"	TP	1.0	96	NA	49	66	10	12
47	A. aerogenes	T	1.2	45	BHI	67	45	10	8
43	"	T	0.9	50	DLA	58	80	0	-
54	"	T	1.7	-	BHI	41	32	14	17
61	"	TP	5.4	45	NA	62	34	23	24
66	"	DKP	5.4	46	NA	42	40	27	19
77	"	DP	6.3	47	T7	56	47	0	0
67	E. aurescens	DKP	1.9	46	NA	51	90	5	-
70	"	DKP	1.4	46	NA	50	35	42	1
117	E. aurescens & B. subtilis	TP	8.5	42	NA	52	50	4***	-
125	E. aurescens & B. subtilis	Sewage	7.0	45	NA	57	37	0***	0
58	E. Freundii	TP	1.9	29	NA	54	40	10	18
62	"	TP	24.0	-	NA	59	73	23	25
65	"	DKP	2.4	44	NA	47	36	11	8
56	"	TP	-	44	NA	57	39	11	7

*See glossary for abbreviations.
**Samples collected with air bubbler off.
***Number of E. aurescens.

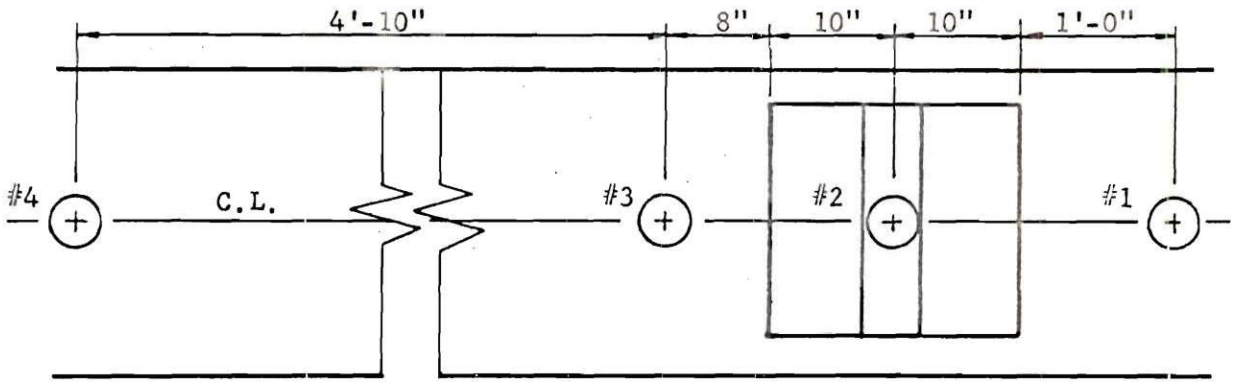
Since coliform concentrations measured at the normal sampling point were small or non-existent, the question arose as to whether aerosols containing the organisms were actually being produced. Experiments were performed to compare the aerosol concentration at a point 8 inches from the downstream edge of the tank with that at the standard point and to trace fallout on settling plates.

The results of upstream versus downstream sampling are summarized in Table 5. Non-coliform organisms show good recovery at both sampling points and a reasonably constant relationship between the two. Escherichia coli were not found at either point, while A. aerogenes may have been present in small numbers, although the colony identification was uncertain. Only E. aurescens was found in significant numbers. However, a high water concentration was required to produce a relatively low concentration in air. Also of interest is the high ratio of downstream to upstream concentration noted for E. aurescens. If the difference is real, the implication would be that the viable organisms occur predominently in the larger droplets which would tend to be screened out by the sample inlet system. Before reaching the downstream point, the size would be sufficiently reduced to show a greater recovery.

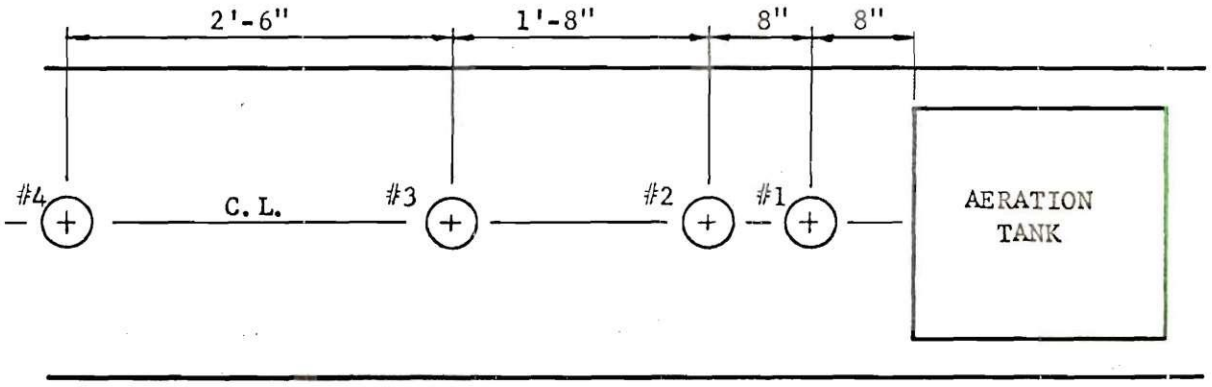
Further searches for viable E. coli and A. aerogenes were made with the aid of settling plates placed on the floor of the tunnel. It was reasoned that these organisms would certainly survive in the larger droplets that would fall to the floor before significant evaporation had occurred. Escherichia coli and S. marcescens were collected on plates distributed as shown in Figure 12a. For E. coli (Exp. No.17) each set



a. Experiments 17 and 19



b. Experiment 79



c. Experiment 81

Figure 12. Settling Plate Distributions

Table 5. Aerosol Concentrations at Upstream and Downstream Sampling Points*

Species	Exp. No.	Liquid	No./ml x10-5	Downstream Conc. No./ft^3	Upstream Conc. No./ft^3	Downstream / Upstream
B. subtilis	95	TP	2.3	4.7	18	0.26
S. salivarius	78	DP	2.0	3.7	8.9	0.42
"	83	DP	3.1	6.2	36	0.17
"	83	DKP	3.1	25	60	0.42
"	84	TP	2.1	13	40	0.33
S. durans	85	DP	4.2	2.8	7.9	0.35
E. aurescens	88	TP	3.5	1.4	2.7	0.52
A. aerogenes	82	DP	1.4	0.2**	0.2**	-
"	82	DKP	1.4	0.3**	0.5**	-
E. coli	76	DKP	1.9	0	0	-

of 2 inch plastic petri dishes containing Endo agar was exposed for ten minutes at air bubble rates of 0.5, 1.0 and 1.5 lpm. In addition, several samples were collected at the downstream sampling point using impingers, slit and Andersen samplers. The experiment was repeated with S. marcescens (Exp. No. 19) except nutrient agar was used and air samples were collected with the slit sampler only. No. E. coli were recovered from samplers or plates. A summary of the recovery of S. marcescens is shown in Table 6.

*See Glossary for abbreviations.
**Colonies resembling A. aerogenes.

Table 6. Recovery of S. marcescens on Settling Plates

Bubble Rate lpm	Total No. of Colonies Found 0 ft	1 ft	3 ft	5 ft	Slit Sampler No./ft^3
0.5	19	11	17	2	9.8
1.0	18	6	9	14	19
1.5	31	39	7	4	29

Aerobacter aerogenes was evaluated in somewhat similar manner. In experiments 79 and 81, 100 mm petri dishes were exposed at the positions shown in Figure 12b and 12c. The results of these experiments are summarized in Table 7. The values shown represent the averages of three 10-minute samples.

Table 7. A. aerogenes on Settling Plates

Exp. No.	Media	Liquid	Point #1	Point #2	Point #3	Point #4
79	Nut.	DKP	0	525	117	1
	DLA	DKP	0	287	84	0
81	Nut.	DP	109	39	5	1
	Nut.	DKP	183	58	6	1

In a separate experiment (No. 80), a further attempt was made to collect A. aerogenes using samplers. At a concentration of 6.0×10^5/ml it was possible at the upstream sampling point to show 1.7 organisms/ft^3 using the Andersen, 3.1 organisms/ft^3 using the slit, and to obtain 10 minute settling plates with too many organisms to count. This was

the only successful recovery of A. aerogenes on samplers out of eight experiments.

If the aerosolization of bacteria by bursting bubbles is purely a mechanical process, it appears quite unusual that viable organisms of the coliform group could not be recovered. The strain of E. coli used had been found by Kethley (59) to be somewhat more sensitive to the airborne state than S. marcescens, ATCC 274, but at least a small number should have survived for one and one-half seconds. Other strains of E. coli have been successfully used in aerosol studies (36, 37, 45, 60, 61) and have shown resistance comparable to S. marcescens (28, 30). It appears that either the bubbling process induces rapid death or that the coliform organisms are absent from the surface and are not aerosolized.

Aerosol Generation as a Function of Concentration

The generation of bacterial aerosols was found to be highly dependent upon the concentration of organisms in the aeration liquid. Such a relationship indicates either a range of droplet sizes was being produced and that increasing concentration increased the probability that the smaller droplets would contain an organism or that only a small percentage of droplets contained bacteria. The relationship was fairly well defined for B. subtilis in tap water plus 100 mg/l peptone, as seen in Figure 13. The data for B. subtilis concentration curves from tap water plus peptone were taken from Experiments 87, 90, 93, 95 and 113, while the DKP curve represents Experiment No. 89.

Large differences were noted in absolute aerosol production rates with different aeration liquids. The magnitude of this difference for

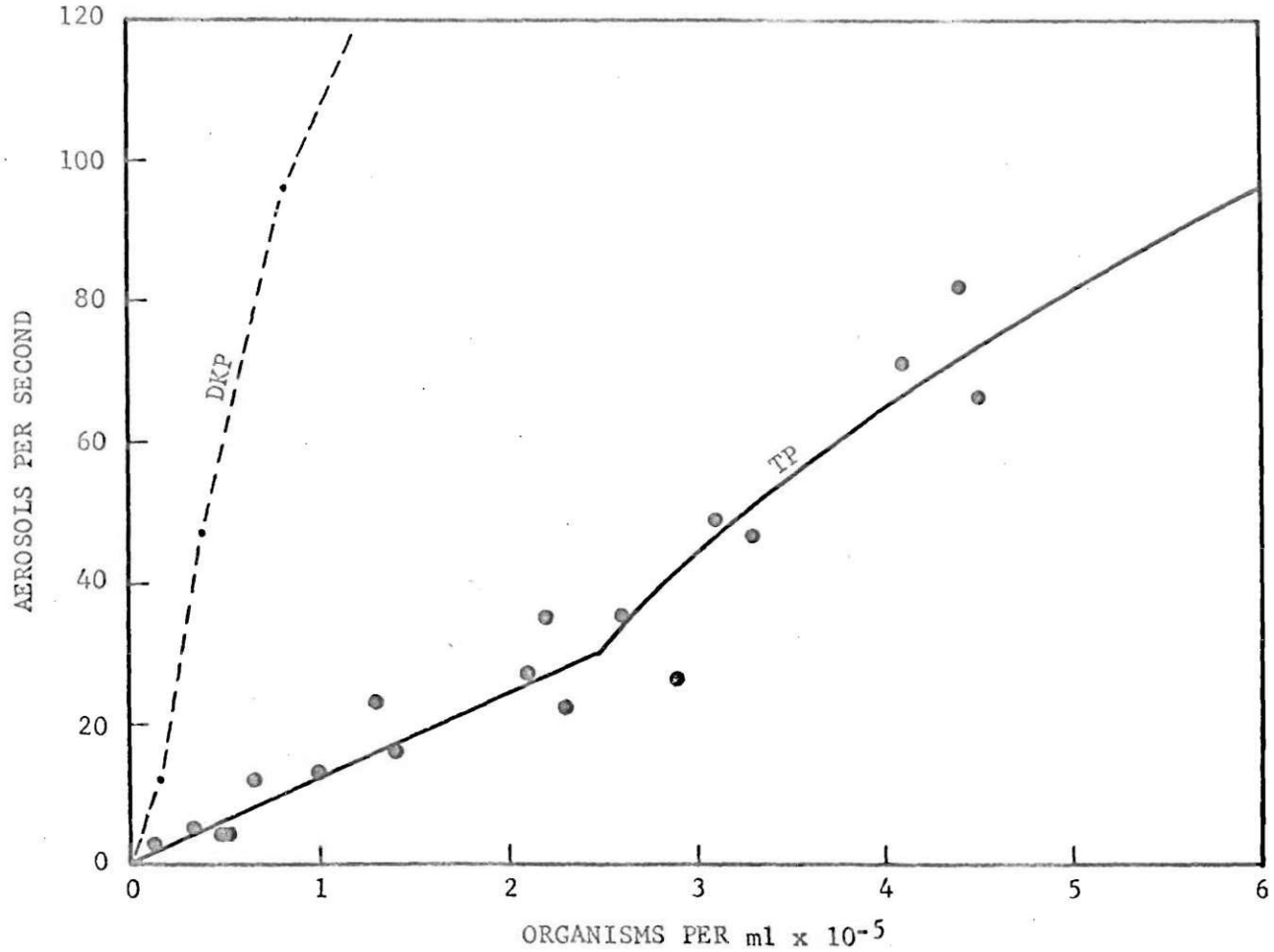


Figure 13. Aerosolization Rate vs. Concentration

B. subtilis from DKP and TP is shown in Figure 13. The production rates of DKP, tap water, and sewage relative to TP may be generalized as 10, 1.5 and 0.2 respectively if TP is taken as unity.

When the data shown in Figure 13 was replotted as R versus log N, as shown in Figure 14, it became obvious that a break occurred at approximately 2.5×10^5 org/ml and the relationship changed from linear at small values of N, to semi-logarithmic at larger values. The straight-line portion of the curve was fitted by the method of least squares, to produce the equation:

$$R = 12.1\ N \qquad (5)$$

for N expressed as no./ml $\times 10^{-5}$.

The 95 per cent confidence interval of the slope is 10.3, 13.9. The semi-logarithmic portion may be defined by the equation:

$$R = 172 \log N - 38.3 \qquad (6)$$

No satisfactory explanation of this change in relationship is advanced. A straight line increase in generation rate was expected up to at least 10^6 cells per ml, with the slope gradually decreasing above this point to become asymptotic to some concentration at which all droplets would contain at least one microorganism. Other liquids and microorganisms failed to show a similar relationship.

The results of several experiments showing aerosol generation rate as a function of concentration are shown in Figure 15. The greater production from demineralized water as compared to tap water is obvious, as is the tendency toward a straight line relationship. The curves

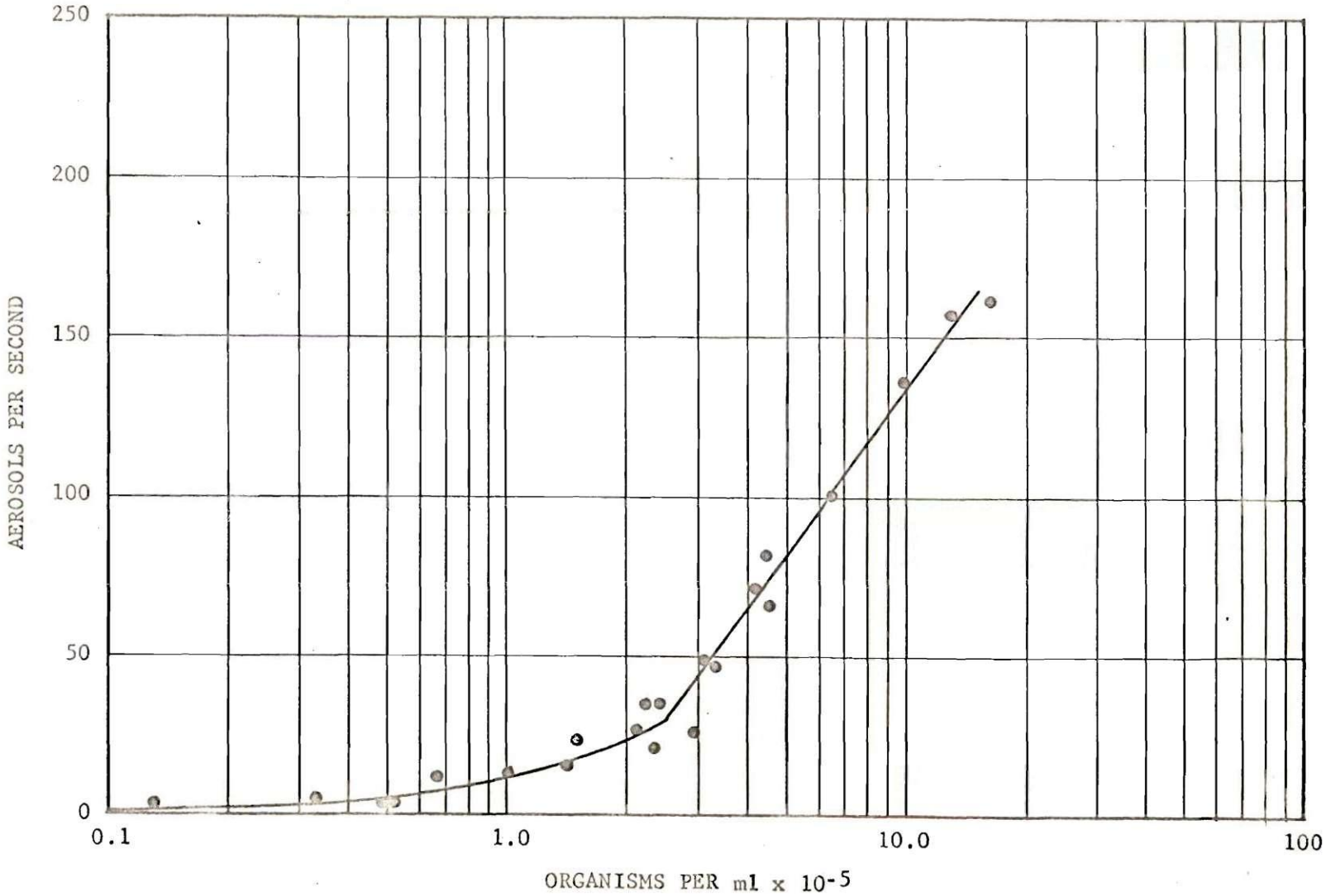


Figure 14. B. subtilis Aerosol Generation Rate

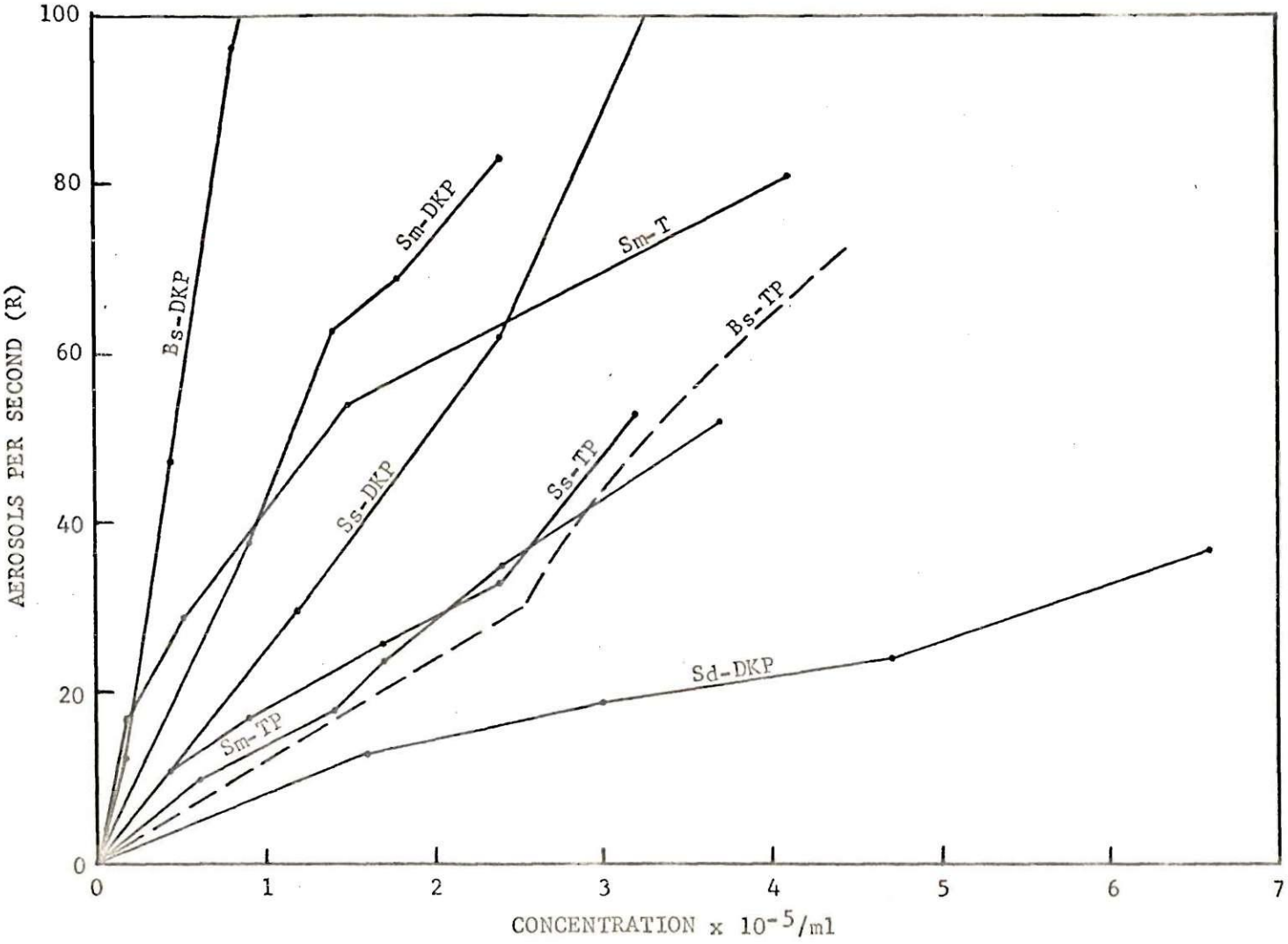


Figure 15. Aerosol Recovery as a Function of Concentration of Organisms

should be regarded only as typical experimental results since it will be shown later that the relative recoveries of species differ from that shown. For example, relative to B. subtilis, the tap water plus peptone curves of S. durans and S. salivarius should be lower, while that of S. marcescens should be higher.

Extreme variations in results were noted in experiments using vegetative species, especially with S. marcescens. Some S. marcescens data are shown in Figure 16. Obviously, it would be impossible to draw meaningful conclusions from such widely dispersed data, even with a very large number of samples. Part of the dispersion is probably due to errors in "N" arising from the use of membrane filters for the determination of number of organisms per milliliter. The major portion, however, appears to be due to the variation in response of the microorganisms and to the influence of the culture upon the surface properties of the liquid. The later effect is illustrated in Figure 17, where B. subtilis concentration data (Figure 13) is plotted along with data obtained when mixed with vegetative species.

The dispersion of data for B. subtilis when mixed with vegetative species cannot be explained on the basis of an error in the number of microorganisms per milliliter. Pour plates were used for enumeration, with the number of colonies of each species being counted separately. The difference in colony color and shape in all cases was sufficient to allow accurate recognition of species. Moreover, a comparison of the number of B. subtilis recovered per milligram of dry spores added showed no significant suppression when other microorganisms were present. The mean yield of all B. subtilis experiments was 9.1×10^3/mg while that of

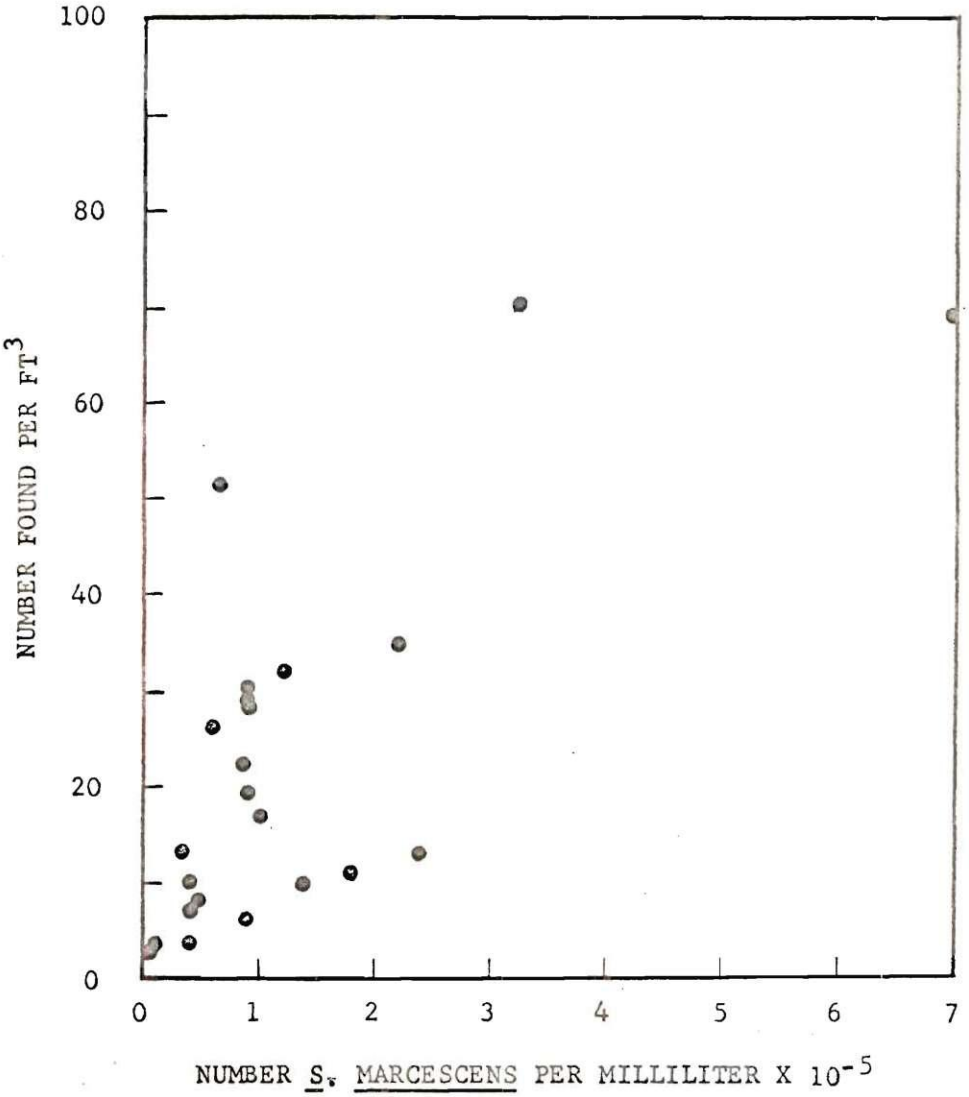


Figure 16. S. marcescens Recovery as a Function of Concentration

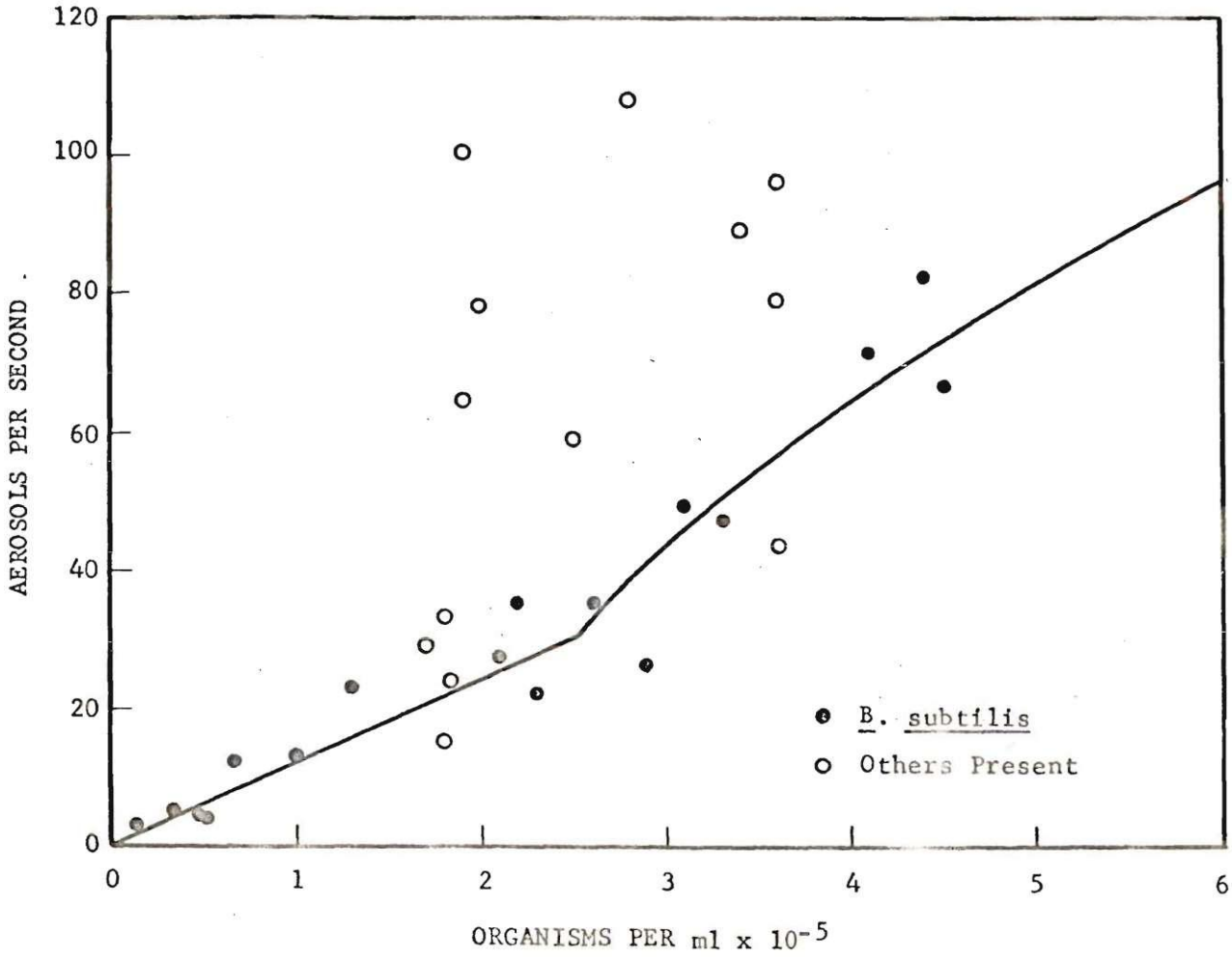


Figure 17. Effect of Other Species on B. subtilis Production

the mixed culture experiments was 8.8×10^3/mg. Little change in B. subtilis generation rate was noted upon the addition of either normal quantities of culture media or a heat-killed culture of S. marcescens. Therefore, the viable organisms or their waste products must exert some influence upon the aerosolization mechanism itself.

As a result of the extreme variability in experimentally determined relationships between viable aerosols recovered and concentration of microorganisms, a series of experiments were performed using B. subtilis and a second species simultaneously in the aeration tank. Such experiments offered the possibility of showing relative yields while minimizing the effects of day-to-day variations. Also advantageous was the possibility of reducing the effect of time of aeration (Figure 11) on viability of the non-spore-formers. Other species were found to have no effect on B. subtilis measured in the air or water; but possible suppression of other organisms by B. subtilis could not be measured. However, colonies developing from aerosol particles containing both B. subtilis and another organism invariably appeared as a normal colony of the vegetative species with a small B. subtilis colored dot in the center. It was assumed that the time lag for germination of the spore allowed the non-sporulating microorganisms to increase to the point that suppression was unlikely.

The results of mixed experiments are shown in Table 8. The abbreviations describing the aeration liquids are as used previously, while K_1 is the concentration of the non-sporulating microorganism found divided by the concentration of B. subtilis, and corrected linearly for differences in number of cells per milliliter. The most striking result was that S. marcescens was apparently generated in much larger numbers

Table 8. Recovery of Several Species Relative to *B. subtilis*

Organisms	Liquid	Experiment No.	Relative Recovery K_1*
S. mars/*B. subt.*	DK	97	1.23
"	DKP	97	1.61
"	DKP	102	1.45
"	T	107	5.04
"	T	112	3.52
"	TP	98a	1.93
"	TP	98b	2.36
"	TP	112	3.27
"	TP	123	2.53
"	TP	124	2.17
"	T+5 mg/l Ivory Soap	107	2.03
"	T+2P	122	2.60
"	Sewage	111	2.95
S. sal/*B. subt.*	DKP	122	0.51
"	TP	99	0.79
"	TP	114	0.22
"	Sewage	110	0.48
S. durans/*B. subt.*	DK	121	0.42
"	DKP	121	0.38
"	TP	96a	0.19
"	TP	96b	0.21
"	TP	118	0.32
E. aures./*B. subt.*	TP	117	<0.01
"	Sewage	125	0.00

than *B. subtilis*, while the other organisms tested showed recoveries as expected.

The mechanism responsible for aerosol generation in the present study is primarily physical. It is subject to the influence of the microorganisms used only through changes in surface characteristics of the liquid. Therefore, the only acceptable explanation of the excessive aerosolization of *S. marcescens* is through the occurrence of larger

* $K_1 = \frac{\text{concentration of vegetative organism}}{\text{concentration of } B.\ subtilis} \times \frac{\text{No. } B.\ subt./ml}{\text{No. veg. Org./ml}}$

numbers of organisms at the surface of the tank than were measured in the bulk of the water. Several experimental observations support this conclusion.

It will be shown later that a range of droplet sizes is produced, and that only a portion of the drops would be expected to contain a bacterium with the number of cells per milliliter typical of these experiments. Therefore, only very few aerosols should contain more than one cell. Table 9 shows the proportion of B. subtilis aerosols recovered which also contained S. marcescens. The proportion varies from 12 to 74 per cent and appears to be fairly independent of the aeration liquid used. In nine experiments employing other species in conjunction with B. subtilis, both species were found in only 0 to 2 per cent of colonies.

In order to verify the suspected abnormal surface concentration of S. marcescens, Andersen samples were taken during Experiment No. 97. The results are shown in Table 10. It may be seen that while the ratio of numbers of the two species was independent of size, the proportion of multiple-organism droplets decreased sharply with reduced diameters. Such a result would be expected if the excess of S. marcescens droplets was due to a higher surface concentration. The smaller droplets have a lesser probability of containing more than one cell simply because of the reduced volume of liquid taken.

It was not possible, with the apparatus available, to determine the cause of concentration of S. marcescens at the air-liquid interface. The reason may have been flotation, the motile cells seeking the oxygen-rich boundary layer, or some form of surface activity. The dependence

Table 9. Proportion of Aerosols Containing Both *B. subtilis* and *S. marcescens*

Run No.	Media	No. containing both species / No. containing *B. subtilis*
97	DK	0.36
	DKP	0.50
102	DKP	0.38
107	T	0.41
112	T	0.22
124	TP	0.38
98a	TP	0.55
98b	TP	0.74
123	TP	0.61
112	TP	0.43
112	T + 2P	0.23
107	T + 5 mg/l Ivory	0.19
111	Sewage	0.12

Table 10. Relative Yields of *B. subtilis* and *S. marcescens* as a Function of Droplet Size

Plate No.	No. *S. mars.* / No. *B. subt.*	No. containing Both Species / No. containing *B. subtilis*
1	2.36	0.44
2	2.20	0.46
3	2.27	0.27
4	2.74	0.06

of recovery ratio upon type of liquid may be seen in Table 8. Tap water produced the highest values of K_1, tap water plus peptone produced intermediate values, while demineralized waters showed low values of K_1. However, the differences may be due to variations in survival after aerosolization and not to different relative generation rates.

The unexpected yields of both S. marcescens and the coliform group may be manifestations of the same phenomenon. Both Webb (28) and Ferry, et al. (30) showed E. coli to survive as long as S. marcescens in air. If the other species of the coliform group may be assumed to have a similar resistance, it is probable that coliforms are repelled from the surface by the same mechanism that allows S. marcescens to be attracted.

If the relative recoveries of S. salivarius and S. durans from Table 8, are averaged, indicated survivals of 50 and 30 per cent, respectively, are obtained. These values are reasonable and tend to indicate that equivalent numbers of droplets containing the Streptococci and B. subtilis are produced. An estimate of the survival of S. marcescens during the approximately 1.5 second period of drying may be taken as no more than 25 per cent (28, 30). Thus, when the relative recoveries of S. marcescens are multiplied by four, the true generation rate may exceed that of B. subtilis by a factor of between 5 and 20. If any of the waterborne pathogens exhibit similar tendencies, a relatively low concentration of pathogens might produce a significant health hazard.

Effect of Composition of Aeration Liquid

In order to estimate the importance of the composition of the aeration liquid upon viable aerosol recovery, a number of experiments were performed during which the composition was varied. Statistically significant changes could be detected in this manner without resorting to large numbers of experiments. The data obtained are shown in Table 11.

Table 11. Effect of Additives

Item No.	Experiment No.	Species	Liquid	R
1	86	B. subtilis	D	2
			DP	15
			DKP	25
2	85	S. durans	DP	19
			DPK	61
3	83	S. salivarius	DP	39
			DPK	152
4	81	A. aerogenes (settling plates)	DP	109
			DPK	183
5	97	B. subtilis	DK	60
			DKP	48
		S. marcescens	DK	86
			DKP	88
6	121	B. subtilis	DK	71
			DKP	92
		S. durans	DK	27
			DKP	31
7	35	S. marcescens	DKP	324
			DK2P	781
8	34	S. marcescens	DKP	659
			DKP + 1 mg/l Antifoam B.	509
9	36	S. marcescens	DKP	175
			DKP + 1 mg/l Antifoam B.	196
10	37	S. marcescens	DKP	130
			DKP + 1 mg/l Antifoam B.	192
11	112	B. subtilis	T	24
			TP	15
			T2P	82
12	90	B. subtilis	TP	26
			TP + 50 ml Boiled S. marcescens	28
13	113	B. subtilis	TP	35
			TP + 30 ml BHI	50
			TP + 60 ml BHI	69
			TP + 90 ml BHI	72
14	107	B. subtilis	T	51
			T + 5 mg/l Ivory Soap	6
		S. marcescens	T	226
			T + 5 mg/l Ivory Soap	11

Table 11. Effect of Additives (cont.)

Item No.	Experiment No.	Species	Liquid	R
15	108	B. subtilis	T	68
			T + 2 mg/l Tide	21
			T + 5 mg/l Tide	16
			T + 10 mg/l Tide	9

Items 1 through 7 show the effects of peptone and K_2HPO_4 upon aerosolization from distilled water. Using B. subtilis, very few organisms were recovered from demineralized water, but sharp increases were noted when peptone and later K_2HPO_4 were added to the water. Either or both peptone and potassium dibasic phosphate were found to extend the life of bubbles and to produce bubble clusters on the surface, while bubbles in demineralized water broke almost immediately. Aerosol generation was thus dispersed over a larger area. Potassium phosphate was much more effective in extending bubble life than peptone. Items 2 through 7 show that adding peptone produces little effect on generation from demineralized water and K_2HPO_4 but the addition of K_2HPO_4 to deminerzliaed water plus peptone results in greatly increased production of aerosols. The reduction in foaming produced by the addition of 1 mg/l of Dow-Corning Antifoam B had little average effect on aerosol concentrations.

In experiments with tap water, it was found that the addition of 100 mg/l peptone resulted in decreased production but if the peptone concentration was raised to 200 mg/l, a large increase occurred in aerosol recovery. At the 200 mg/l level of peptone, very large numbers of small

bubbles produced a mat over much of the surface of the tank. Monro and Yatabe (62) found that 200 mg/l peptone was the lower concentration limit for production of a stable foam on tap water plus one per cent sewage.

Photographs of bubbles produced by a medium bubbler operated at 0.5 lpm in D, DP, DKP, T and TP are shown as Figures 18, 19, 20, 21 and 22, respectively. Aerosol dissemination was not entirely a function of bubble stability, since tap water produced aerosols at a higher rate than tap plus peptone.

That production rates depended more upon bubble stability than surface tension was verified by the addition of Ivory Soap and Tide detergent to tap water. Bubble life was reduced sharply at low concentrations of either material and the reduction in aerosol recoveries may be seen from Items 14 and 15, Table 11. Dibasic potassium phosphate, at 160 mg/l, was found to produce no change (7 trials) in the surface tension of demineralized water at 24°C.*, while 100 mg/l peptone produced a drop from 72.0 dynes/cm to only 69.7 dynes/cm (8 trials). Thus, the changes in generation rates observed must be attributed to bubble stability. The present data did not show whether the higher rate depended upon the presence of clusters only, or whether the clusters merely allowed a larger portion of the surface of the tank to be effective. Inferences on the mechanism are presented in the following section based on size data.

The effects of culture media are shown as Items 12 and 13, Table 11. The addition of 50 ml of a 48-hour culture of S. marcescens brought to an impending boil to destroy the microorganisms had no affect on generation

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* Du Nouy Interfacial Tensiometer, Model No. 3565.

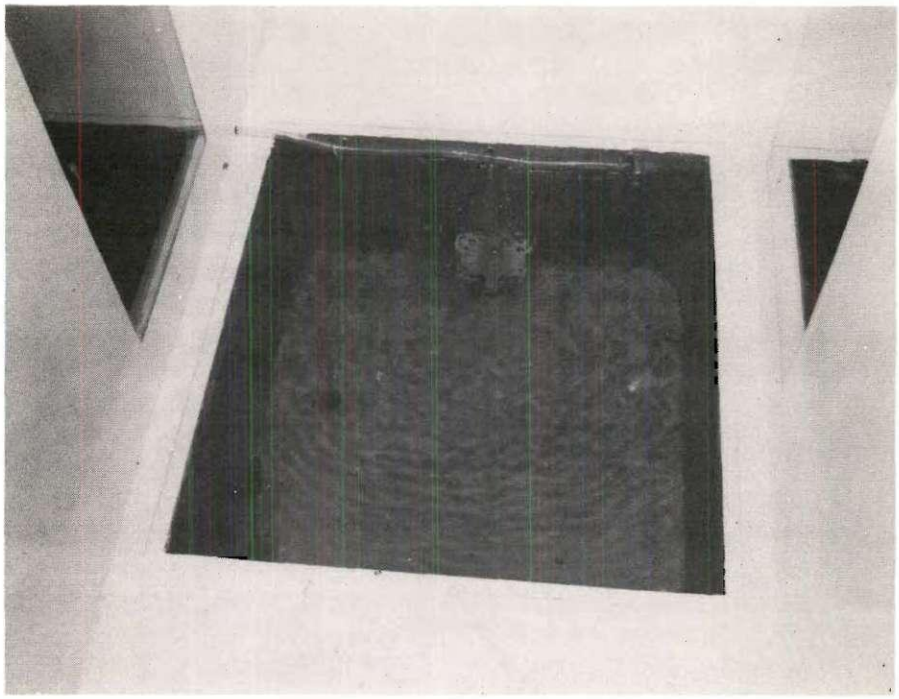

Figure 18. Bubble Pattern Using Demineralized Water

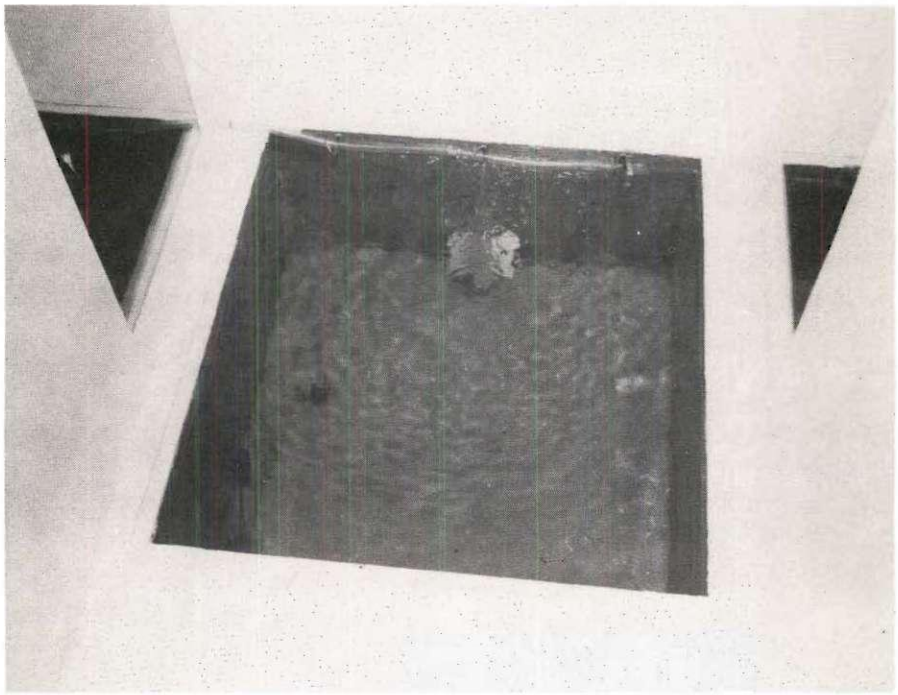

Figure 19. Bubble Pattern Using Demineralized Water Plus 100/1 Peptone

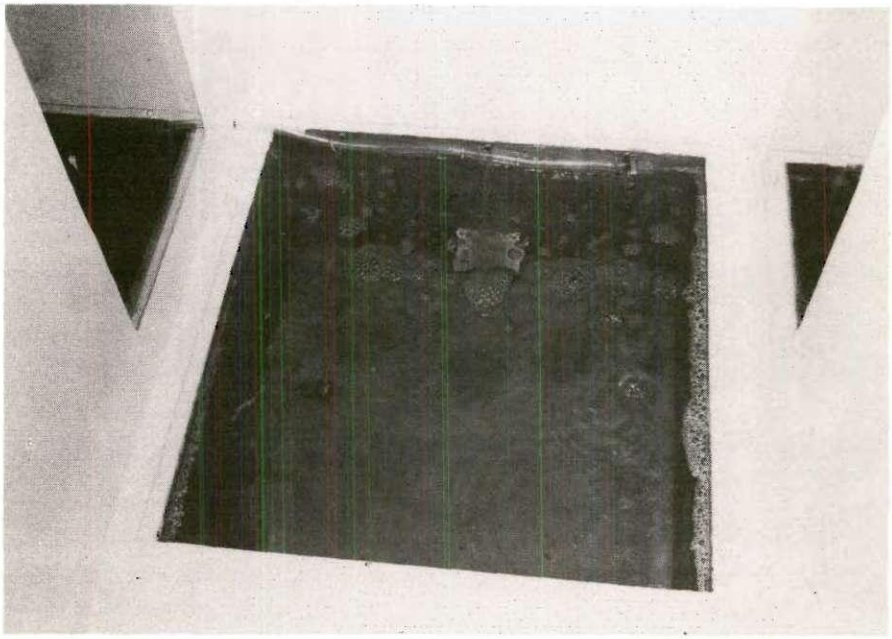

Figure 20. Bubble Pattern Using Demineralized Water Plus 100 mg/l Peptone and 160 mg/l K_2HPO_4

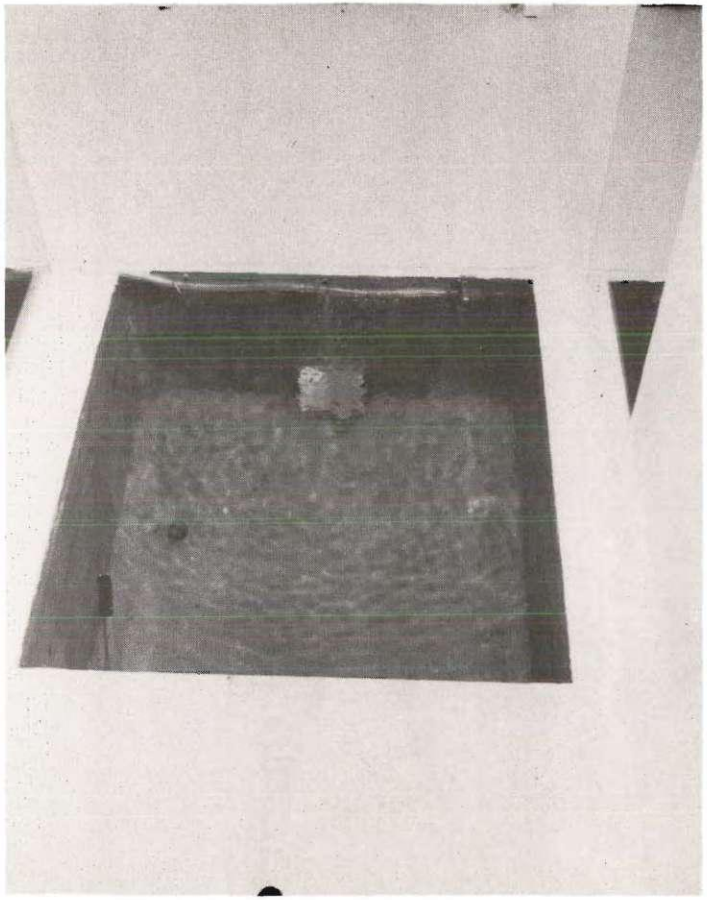

Figure 21. Bubble Pattern
Using Tap Water

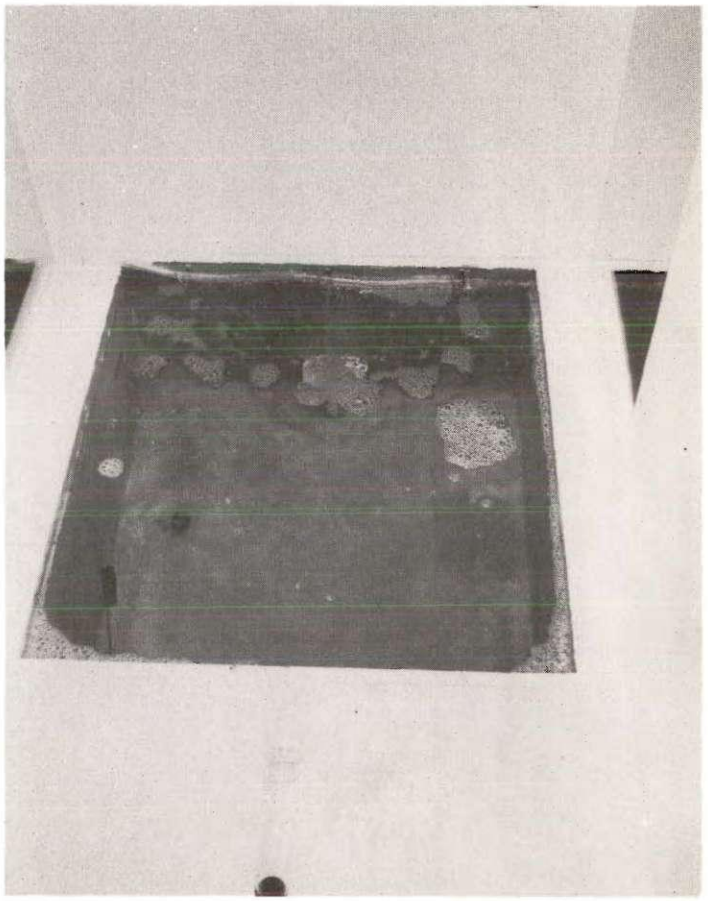

Figure 22. Bubble Pattern Using Tap
Water Plus 100 mg/l Peptone

rate. However, freshly prepared Brain Heart Infusion Broth caused an increase in recovery. As with peptone, bubble stability was increased and the mechanism was presumed the same.

Droplet Size

The diameters of residues remaining after evaporation of droplets are important in determining the possible route and potential for infection. Residues greater than five microns in diameter would be expected to be largely retained in the upper respiratory system, while smaller particles might reach the alveoli (18). The relative importance of the two areas of deposition cannot be defined for the present study because of the wide variety of pathogenic organisms that might be encountered in polluted waters. Smaller particles would be of importance when produced from an extended aeration sewage treatment system at a tuberculosis sanitorium, while larger droplets containing Salmonella typhosa might be critical when deposited in the nose and throat.

Size distribution curves of evaporated residues obtained when B. subtilis and S. marcescens were aerosolized from tap water plus 100 mg/l peptone and demineralized water plus 100 mg/l peptone and 160 mg/l K_2HPO_4 are shown as the lower group in Figure 23. Estimations of original droplet sizes for B. subtilis from TP and DKP are shown as the upper curves in Figure 23. The assumptions of complete evaporation of water and unit density of solids were used in calculation of original sizes. Thus, the curves must be considered as upper limits on droplet sizes, and may actually fall as much as 10 per cent lower.

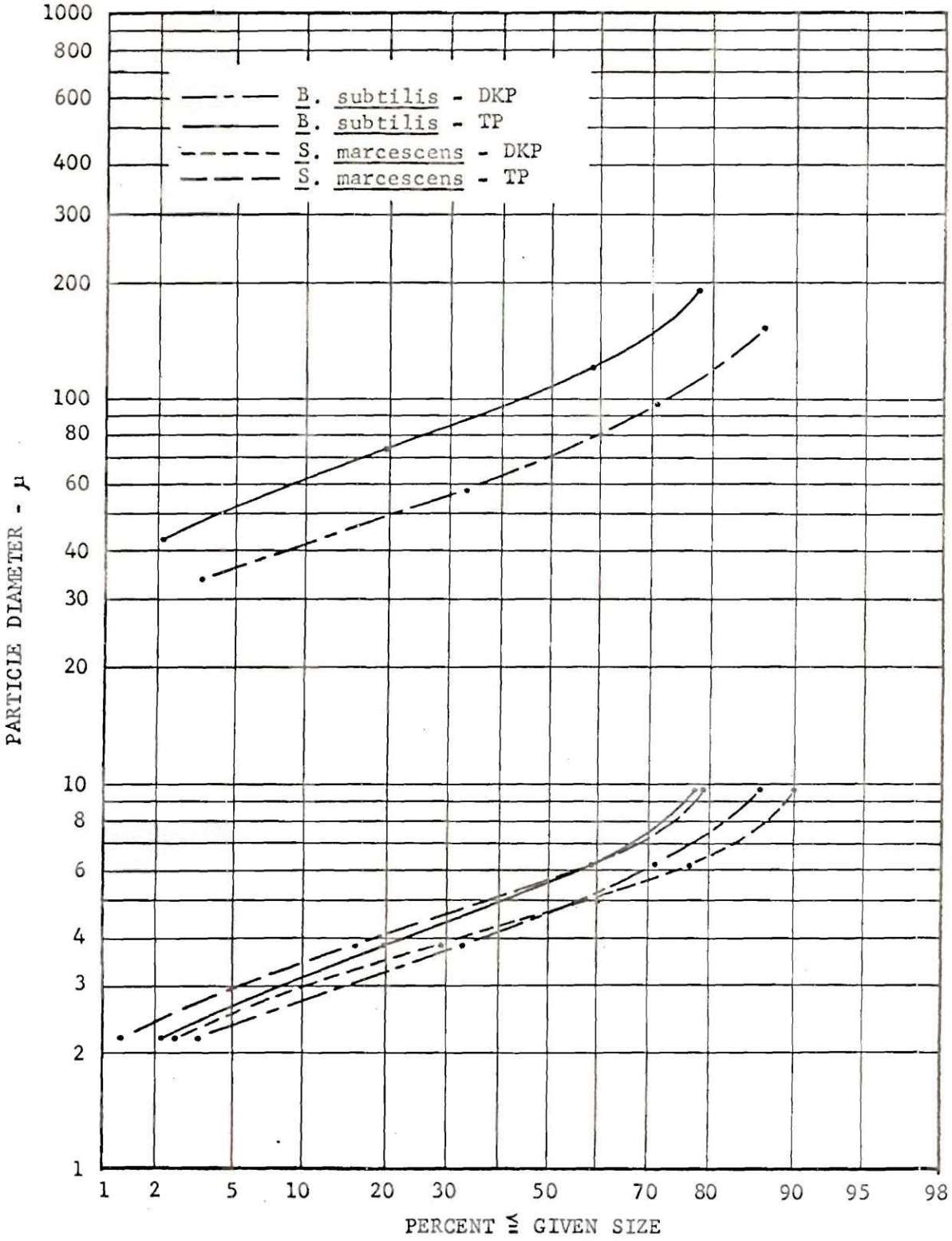

Figure 23. Calculated Sizes of Droplets Produced

The curves are linear for the smaller sizes and essentially parallel, but TP (130 mg/l solids) was expected to produce smaller particles than DKP (260 mg/l solids). The difference is too great to be the result of a higher equilibrium moisture content in the tap water solids and must be attributed to a difference in original droplet sizes produced. The departure from a straight line indicates incomplete evaporation of the larger droplets. Using Woodcock's (3) estimate that droplet diameter is 10 per cent of the air bubble diameter, most jet-droplets should exceed 100 μ diameter. This corresponds approximately with the largest size droplet that would evaporate completely in the 1.5 second time interval between aerosolization and collection.

A slightly excessive number of large particles is quite significant in a sampling system which discriminates against them. May (63) found that only four per cent of particles of 30 μ diameter and 13 per cent of particles with a 20 μ diameter were collected by the Andersen sampler when used with the upper cone. In the present work, not only the cone, but a bent intake tube was employed. Thus, it appears that a population of very large particles exists and only a few were sampled.

It is interesting to note that since some solids were added along with the culture of S. marcescens, the curves should lie to the right of the B. subtilis curve (see Figure 23) for the same medium. However, this occurs only for larger droplets. The cells in smaller particles are exposed to greater destructive forces and points are actually found to the left of B. subtilis below about five microns diameter.

The measured diameter of droplet residues was found to vary with the solids content of the liquids, indicating that the size distribution

before evaporation of droplets was reasonably independent of type of liquid media. Measured sizes of droplet residues from four different liquids are shown in Figure 24.

The initial size distribution of droplets recovered extends below the range expected from jet-droplets alone, but is much too large for film-droplets (2). The dependence of aerosol generation rate upon the presence of bubble clusters has been shown earlier and suggests a third mechanism of formation. Before a bubble bursts, the film drains and becomes thinner (3). For a single bubble, the drainage returns to the bulk of the liquid, but in clusters, the drainage may be trapped at the point of junction of the films of adjacent bubbles. A thickening of the film at this point would provide a source of droplets intermediate in size between those from the film and those from jet droplets.

It is of interest to compare the concentrations of microorganisms used with those required to provide an average of one microorganism per droplet. Taking the median diameter of original droplets from DKP as 72μ, each 2.0×10^{5} μ^3 should contain at least one organism. If the cells were uniformly distributed, 5×10^{6} per milliliter would be required. The concentrations used were typically less than 10 per cent of this value, indicating that potential production of aerosols was far greater than that measured.

Health Hazards

The results show that large numbers of airborne microorganisms may be produced by bubbling air through water. It is almost inconceivable however, that natural waters could contain a sufficient concentration of

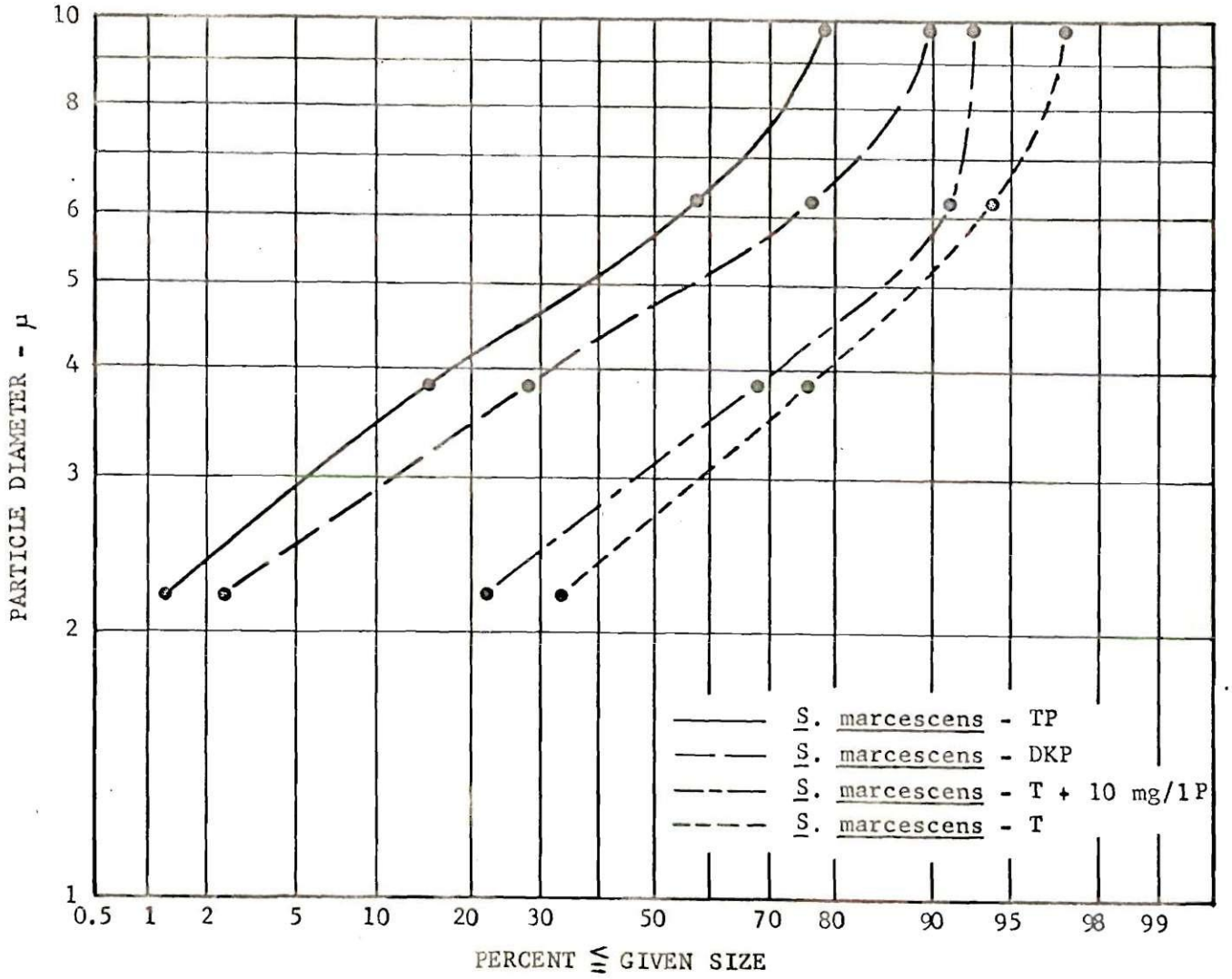

Figure 24. Size Distribution of Aerosols Recovered

pathogenic microorganisms to produce a significant hazard. Sewage, on the other hand, may contain a sufficient number of pathogenic bacteria, or viruses, particularly during an epidemic or when hospital wastes are involved, to produce a significant threat in the vicinity of the treatment device. If any of the pathogens are concentrated at the surface of the sewage, as is S. marcescens, the number aerosolized could be quite substantial. Until further information becomes available, sewage aeration should be regarded as potentially hazardous to persons in the immediate vicinity.

CHAPTER V

CONCLUSIONS

The production of viable bacterial aerosols by bursting bubbles is highly dependent upon species. Using B. subtilis as a basis for comparison, excess numbers of S. marcescens, comparable numbers of the Streptococci and a pronounced deficiency of coliform organisms were noted. The data suggests that the non-uniform production of aerosols is due to a difference in concentration of the cells at the surface from that in the bulk of the aeration liquid. The difference may be either positive or negative and is highly dependent upon both species and composition of the aeration liquid.

The coliform group of bacteria may not be a reliable index of bacterial air pollution resulting from sewage aeration. The very low aerosolization rate of coliform organisms precludes the conclusion that the absence of members of the group indicates an absence of pathogenic bacteria in air. Until information becomes available on the aerosolization of pathogens, it is suggested that a total colony count may be a more appropriate measure of air contamination by aeration processes.

Calculated original diameters of droplets produced by bursting bubbles indicated that many particles were too small to have originated through the jet-droplet mechanism. A possible source may be the thickened film at the point of juncture of adjacent bubbles.

In addition to the primary variables of species and composition of aeration liquid, other variables affecting the aerosolization process were surface tension, bubbling rate, bubble size, wind velocity, concentration of cells, and physiological state of the culture. The effects of air and water temperature or of relative humidity could not be isolated from the data. The large number of variables affecting the net aerosolization rate indicates that a realistic evaluation of the bacterial air pollution resulting from an aeration process should require large volume air samples taken over an extended period of time.

CHAPTER VI

RECOMMENDATIONS

One of the more interesting and important topics requiring further investigation is the increased concentration of S. marcescens at the surface of the aeration tank. However, unless the underlying reason is simply floatation, which appears unlikely from the present work, a conclusive explanation could be technically difficult. Based on the work of Ferry, et al. (30), Webb (28), and others, at least 10 per cent of E. coli should have survived aerosolization. It is possible, therefore, that the same mechanism that produced large surface concentrations of S. marcescens caused the coliform group to migrate from the surface into the bulk of the liquid.

In conjunction with an effort to explain the apparent "surface activity" of S. marcescens, the present experiments should be extended to include some of the harmful organisms known to be present in sewage. These need include only the more common organisms such as Staphlococci, some viruses, and representatives of groups causing gastro-intestinal disorders. Yields of these organisms should be compared with a reference spore to detect any possible surface concentration. The more exotic disease organisms could be omitted since it is very unlikely that sufficient concentration would occur in sewage to produce any significant number of aerosols. A small, totally enclosed system using fans to support and mix the aerosols and an aeration tank at the bottom would

be sufficient for comparative purposes and could be disinfected with steam.

In addition to the laboratory studies, measurements such as those performed by Albrecht (5) at a trickling filter plant should be conducted at a number of sewage aeration plants. Because of the difficulty in collecting and identifying a particular group of organisms, a wide spectrum media such as brain heart infusion agar should be used. Assay of the sewage using pour plates of the same agar would allow at least an approximation of the relationship between number per milliliter and number of aerosols recovered.

A great deal of variation in aerosol concentration with time would be expected at a particular time and also between plants. For this reason, large numbers of samples should be collected. Interpretation of results would be simplified with a network of sampling points at a constant time-distance downwind and at several heights above the ground. The factors of death rate and verticle dispersion could thus be eliminated, and the results could be expressed in terms of total apparent generation rate of the entire unit. Sample collection could be greatly simplified through the use of slit-tray samplers as described by Decker, et al. (64). A battery of these samplers installed upwind and downwind from the aeration unit could be put in place and operated up to eight hours without change in media. The data would be quite reliable and preparation simplified, but the initial cost would be high.

APPENDIX A

ANDERSEN SAMPLER CORRECTIONS

Table 12. Corrections for Counts Using the Andersen Sampler

No. Positive Holes	Corrected Count	No. Positive Holes	Corrected Count
10	10	190	258
20	21	200	277
30	31	210	298
40	42	220	319
50	53	230	342
60	65	240	367
70	77	250	392
80	89	260	420
90	102	270	450
100	115	280	482
110	129	290	516
120	143	300	555
130	157	310	597
140	172	320	644
150	188	330	697
160	204	340	759
170	221	350	832
180	239	360	921

APPENDIX B

DATA SHEETS

Exp. #_____

Date_____

_____

_____

Outside Temp._____°F.

Rel. Humidity_____ %

Culture_____

_____

Dose_____ml.

Age of culture_____hours

Bubbler_____

Intake_____mm=_____in.

Tank Temperature_____°F.

Tank pH_____

Tank Liquid_____

_____

_____

Collection Media_____

Samplers_____

Notes:_____

_____

_____

_____

_____

_____

_____

_____

Exp. #_____

Date_____

Page_____of_____

Experiment No._____

Start Time A_____

B_____

C_____

Length of Run A_____min

B_____min

C_____min

_____ A_____

_____ B_____

C_____

Water Analyses A_____

_____ B_____

C_____

Bubble Flow Rate A_____lpm

B_____lpm

C_____lpm

Feet of Air A_____

B_____

C_____

Wind Velocity A_____fps

B_____fps

C_____fps

Start Incub._____

Stop Incub._____

Sample No.	Org. Found	Corr. Count	Notes

APPENDIX C

SLIT SAMPLER DATA

The following table contains the results of all experiments in which the slit sampler was used. Column headings and conventions which were used to condense the data are as shown below.

1. Column headings are explained as follows:

   a. Exp. No. - experiment number.

   b. Spec. - Species of bacteria (See glossary for abbreviations).

   c. Cult. Age (hrs) - The time of incubation, in hours, allowed for growth before the bacteria were introduced into the aeration tank.

   d. Media - Type of agar used for sample collection. Abbreviations are:

      TGEK - Tryptone glucose extract agar with 5 gm/l sodium chloride and 2.5 gm/l dibasic sodium phosphate.

      EA - Endo agar.

      NA - Nutrient agar.

      BHA - brain heart infusion agar.

      VRB - Violet red bile agar.

      DLA - Desoxycholate lactose agar.

      MSA - Mitis salivarius agar.

      T7 - Tergitol 7 agar.

      ENA - Enterococcus agar.

PRS - Phenol red sacchrose agar.

TSA - Trypticase soy agar.

e. $Nx10^{-5}$ - Number of bacteria per milliliter $x10^{-5}$.

f. Liquid - Type of aeration liquid (See glossary of abbreviations for symbols).

g. n - Number of samples.

h. R - Number of aerosols per second passing the sampling point.

i. R.H.(%) - Relative humidity in per cent.

j. Air temp. (^{o}F) - Temperature of air in ^{o}F measured at the mouth of the tunnel.

2. Items in a column are assumed to apply for subsequent sample groups until a change is noted, except R.H. and Air temp. which are always listed with the sample group to which they apply. For example, in Experiment No. 17, Escherichia coli was used throughout the experiment with 1.7×10^{5}/ml present for the first 3 samples and 2.3×10^{-5} present during collection of the last 5 samples. In Experiment No. 19, the R.H. and Air temp. were measured as the first and last sample groups were taken, but no measurement was made during collection of the second group.

3. Two types of notes are used. Special notes applying to a particular sample or sample group and general notes applying to the entire experiment. Special notes are shown at the left hand side of the note column and in line with the sample to which they correspond. In Experiment No. 17 the air bubble flow rate in liters per minute illustrates the use of special notes. General notes are always numbered (even if only one is used). Experiment No. 17 has 3 general notes.

4. The standard sample was defined as having the following characteristics:

a. One medium bubbler used.

b. Air bubble flow rate is 0.5 lpm.

c. Sample collection at downstream point.

d. Sampling point 2.25 inches above the floor.

e. R corrected for wind velocity other than 4.1 fps.

f. Samples taken consecutively with no delay between groups shown.

g. Water analyses by pour plate method using same media used in sample collection.

5. Notes are shown only when conditions deviate from standard.

6. For experiments in which two species were used simultaneously, two entries are required for each sample group to show N and R for each species. The species to which N and R apply are shown in special notes. Experiment No. 96 illustrates the use of 6 lines to show the results of 3 groups of 3 samples each.

Table 13. Slit Sampler Data

Exp. No.	Spec.	Cult. Age (hrs)	Media	N x10^{-5}	Liquid	n	R	R.H. (%)	Air Temp. (°F)		Notes
17	Ec	73	TGEK	1.7	DKP	1	0			0.5 lpm	(1) Air bubbling rate varies as shown. (2) MF water analysis. (3) 2-med. bubblers.
						1	0			1.0 lpm	
						1	0			1.5 lpm	
			EA	2.3		1	0			0.5 lpm	
						3	0			1.0 lpm	
						1	0			1.5 lpm	
19	Sm	47	TGEK	8.9	DKP	3	94	20	77	0.5 lpm	(1) Air bubbling rate varies as shown. (2) 2 coarse bubblers. (3) MF water analysis.
						3	186			1.0 lpm	
						3	284	18	78	1.5 lpm	
20	Sm	47	TGEK	0.78	DKP	3	300			0.5 lpm, 4.2 fps	(1) Air bubbling rate and wind velocity varies as shown. (2) MF water analyses. (3) 2 medium bubblers.
						3	344			1.0 lpm, 2.8 fps	
						2	538			1.0 lpm, 3.2 fps	
						3	412			1.0 lpm, 5.3 fps	

Table 13. Slit Sampler Data (cont.)

Exp. No.	Spec.	Cult. Age (hrs)	Media	N x10^{-5}	Liquid	n	R	R.H. (%)	Air Temp. (°F)	Notes
21	Sm	46	TGEK	0.96	DKP	3	75			(1) Air bubbling rate = 1.0 lpm. (2) 2 coarse bubblers. (3) MF water analyses.
				2.0		2	100			
				4.1		3	143			
30	Sm	73	TGEK	5.7	DKP	2	184	54	61	H = 0" (1) Height of intake varied as shown. (2) MF water analyses.
						2	454			H = 1-1/2"
						2	149			H = 3-5/8"
						2	19			H = 8"
						2	8			H = 12-1/8"
31	Sm	47	TGEK	6.0	DKP	3	569	61	55	H = 0" (1) Height of intake varies as shown. (2) MF water analyses.
						3	175			H = 6-1/4"
						3	TNTC			H = 1-3/8"
33	Sm	46	NA	7.0	DKP	5	445	61	55	(1) MF water analyses.
34	Sm	49	NA	4.8	DKP	5	659	40	57	(1) MF water analyses. (2) Add 1 mg/l Antifoam B before second sample group.
						5	509			
35	Sm	49	NA	0.64	DKP	3	324	40	62	(1) MF water analyses.

Table 13. Slit Sampler Data (cont.)

Exp. No.	Spec.	Cult. Age (hrs)	Media	N x10^{-5}	Liquid	n	R	R.H. (%)	Air Temp. (°F)	Notes
35 (cont.)	Sm				DK2P	4	781			
36	Sm	47	NA	0.91	DKP	5	175			(1) MF water analyses. (2) Add 1 mg/l Antifoam B before second sample group.
						5	196	61		
37	Sm	48	NA	0.86	DKP	3	130	81	75	(1) MF water analyses. (2) Add 1 mg/l Antifoam B before second sample group.
						3	192			
38	Sm	25	NA	0.42	DKP	3	65	84	59	(1) MF water analyses. (2) Aeration liquid and culture changed between sample groups.
		25			DKP	3	24	100	55	
		25			DKP	3	46	94	53	
39	Sm	39	NA	0.90	DKP	2	125	50	49	(1) MF water analyses. (2) Aeration liquid changed between sample groups, but portions of same culture used.
					DKP	3	186	39	53	
					DKP	3	195	32	58	
40	Sm		NA	0.03	DKP	3	17	40	62	(1) MF water analyses. (2) More of culture added between sample groups.
				0.08		3	22	41	64	
				0.33		3	86	38	66	
				0.59		3	169	38	66	

Table 13. Slit Sampler Data (cont.)

Exp. No.	Spec.	Cult. Age (hrs)	Media	N x10^{-5}	Liquid	n	R	R.H. (%)	Air Temp. (°F)	Notes	
40 (cont.)				1.2		3	206	38	64		
41	Ec	46	BHA	0.26	DKP	3	0	58	50	(1) Wind velocities from 5 to 6 fps.	
			VRB			3	0				
			BHA			3	0				
43	Aa	50	DLA	0.88	T	3	0	80	58		
44	Sm	48	NA	0.22	T	3	53	82	59	t = 0	(1) MF water analyses.
						3	37	80		t = 1.15 hrs.	(2) Times from midpoint of first
						3	38	74	61	t = 2.42 hrs.	sample group to midpoint of others
						3	33	66	62	t = 3.67 hrs.	as shown.
						3	31	58	64	t = 5.38 hrs.	
45	Sm	48	NA	0.22	T+0.1P	3	33	50		t = 0	(1) MF water analyses.
						3	27		62	t = 1.18 hrs.	(2) Times from midpoint of first
						3	29			t = 2.57 hrs.	sample group to midpoint of others
						3	26	45	64	t = 4.08 hrs.	as shown.

Table 13. Slit Sampler Data (cont.)

Exp. No.	Spec.	Cult. Age (hrs)	Media	N x10^{-5}	Liquid	n	R	R.H. (%)	Air Temp. (^{o}F)	Notes	
46	Sm	45	NA	0.30	TP	3	65	58	62	t = 0	(1) MF water analyses.
						3	52	58	68	t = 1.78 hrs.	(2) Times from midpoint of first sample group to midpoint of others as shown.
						3	26	79	65	t = 4.03 hrs.	
						3	16	72	65	t = 5.82 hrs.	
						3	17	72	63	t = 7.58 hrs.	
						3	18		61	t = 9.18 hrs.	
47	Aa	45	BHA	1.2	T	2	3.6	45	67	0 lpm	
						3	4.4			0.5 lpm	
						3	0.4			0.5 lpm (overlay with VRB after 3 hrs incub.).	
						3	0.9			0.5 lpm (overlay with VRB before incub.).	
48	Sm	42	NA	0.01	TP	3	6.0	94	45	t = 0	(1) Times from midpoint of first sample group to midpoint of others as shown.
						3	6.4	86	42	t = 2.63 hrs.	
						3	3.7	84	37	t = 7.85 hrs.	
						3	1.6	65	32	t = 16.2 hrs.	
						3	2.7	100	35	t = 22.3 hrs.	

Table 13. Slit Sampler Data (cont.)

Exp. No.	Spec.	Cult. Age (hrs)	Media	N x10^{-5}	Liquid	n	R	R.H. (%)	Air Temp. (^{o}F)	Notes		
										t	N_{MF}	N_R
50	Sm	47	TGEK		TP	3	29	77	53	0	0.38	0.42
						3	25			1.20 hrs.	0.35	0.36
						3	18			3.03 hrs.	0.29	0.33
						3	8.6			5.28 hrs.	0.40	0.35
						3	3.7			22.2 hrs.	0.80	0.45
						3	4.3			25.7 hrs.	1.20	0.75
51	Ec	44		1.77	TP	3	3.0		25	BHA, 0 lpm.		
						3	3.9			BHA, 0.5 lpm.		
						2	1.3			BHA/VRB overlay, 0.5 lpm.		
						2	0			VRB, 0.5 lpm.		
						2	0			DLA, 0.5 lpm.		
						2	0			DLA/DLA overlay, 0.5 lpm.		
54	Aa	49	BHA	0	T	4	6.8	32	41			
				1.7		4	6.0					
55	Sm	42	NA	0.19	T	3	10	32	44	0.25 lpm (1) MF water analyses.		

Table 13. Slit Sampler Data (cont.)

Exp. No.	Spec.	Cult. Age (hrs)	Media	N x10^{-5}	Liquid	n	R	R.H. (%)	Air Temp. (°F)	Notes
55 (cont.)						3	9	40	54	0.50 lpm
						3	17			0.75 lpm
						3	20			1.0 lpm
						3	30			1.5 lpm
						3	64			2.0 lpm
56	Ef	44	NA		T+0.5P	4	4.7	39	57	0.5 lpm
						4	3.0			0.0 lpm
57	Ss	45	MSA	0.58	TP	6	0.6	56	40	(1) Water analyses by MF on MSA.
58	Ef	29	NA	1.9	TP	4	4.5	40	54	0.5 lpm
						3	7.7			0.0 lpm
59	Ss	51	MSA	1.2	T	5	0.2	38	54	(1) Water analyses by MF on MSA.
					TP	5	1.7		52	
60	Ec	96	NA	1.0	TP	5	4.3	66	49	0.5 lpm
			T7			5	0			0.5 lpm
			NA			5	5.1			0 lpm

Table 13. Slit Sampler Data (cont.)

Exp. No.	Spec.	Cult. Age (hrs)	Media	N $x10^{-5}$	Liquid	n	R	R.H. (%)	Air Temp. (°F)	Notes
61	Aa	45	NA	3.0	TP	5	9.8	34	62	0.5 lpm
			NA			5	10.3			0 lpm
			T7			5	0			0.5 lpm
62	Ef	45	NA	1.9	TP	5	9.8	73	59	0.5 lpm
			NA			5	10.7			0 lpm
			T7			5	0			0.5 lpm
63	Sm	45	NA	0.66	TP	3	41	89	62	(1) MF water analyses.
						3	55			
						3	52			
64	Ss	46	MSA	0.90	DKP	5	0	73	32	0.5 lpm (1) Water analyses by MF on MSA.
						5	0			0 lpm
65	Ef	44	NA	2.4	DKP	4	4.7	36	47	0.5 lpm
			NA			4	3.4			0 lpm
			T7			4	0			0.5 lpm
66	Aa	46	NA	5.4	DKP	4	12	40	42	0.5 lpm

Table 13. Slit Sampler Data (cont.)

Exp. No.	Spec.	Cult. Age (hrs)	Media	N x10^{-5}	Liquid	n	R	R.H. (%)	Air Temp. (^{o}F)	Notes
66 (cont.)			NA			4	8.1			0 lpm
			T7			3	1.3			0.5 lpm
67	Ea	46	NA	1.9	DKP	3	3.0			(1) Approx. 2 hr.-45 min. between sample means.
						3	1.3			
70	Ea	46	NA	1.4	DKP	3	18	35	50	0.5 lpm
			NA			3	0.3			0 lpm
			DLA			3	0			0.5 lpm
71	Sd	47	BHA	0.27	DKP	3	8.5	80	53	0.5 lpm (1) Water analyses by MF on ENA.
			BHA			3	3.9			0 lpm
			ENA			3	0			0.5 lpm
72	Ss	48	BHA	0.62	DKP	3	3.0	38	34	0.5 lpm (1) Water analyses by MF on MSA.
			BHA			3	26			0 lpm
			MSA			3	0.9			0.5 lpm
73	Sm	48	NA	0.18	T	3	17	40	39	(1) MF water analyses.

Table 13. Slit Sampler Data (cont.)

Exp. No.	Spec.	Cult. Age (hrs)	Media	N x10^{-5}	Liquid	n	R	R.H. (%)	Air Temp. (°F)	Notes
73 (cont.)				0.51		3	29			
				1.5		3	54			
				2.6		3	81			
74	Sm	44	NA	0.39	T	3	16	94	43	(1) MF water analyses.
				1.1		3	32			
76	Ec	49	T7	1.9	DKP	4	0	33	46	Upstream
						4	0			Downstream
77	Aa	47	T7	6.3	DP	4	0	47	56	Upstream
						3	0			Downstream
78	Ss	45	PRS	2.0	DP	3	57	40	66	Upstream
						3	24			Downstream
80	Aa	45	DLA	6.0	DKP	3	20	49	46	Upstream
82	Aa		NA	1.3	DP	3	1.3		33	Upstream
			T7			3	0			Upstream
			NA			3	1.3			Downstream

Table 13. Slit Sampler Data (cont.)

Exp. No.	Spec.	Cult. Age (hrs)	Media	N x10-5	Liquid	n	R	R.H. (%)	Air Temp. (°F)	Notes	
82 (cont.)			NA		DKP	3	1.7			Upstream	
			NA			3	3.0			Downstream	
83	Ss	41	PRS	3.2	DP	3	234	60	38	Upstream	
						3	39			Downstream	
					DKP	3	TNTC			Upstream	
						3	152			Downstream	
84	Ss		PRS	0	TP	3	0	42	44	Downstream	(1) Approx. $3\frac{1}{2}$ hrs. between sample groups 3 and 4.
				2.1		3	254			Upstream	
						3	78			Downstream	
						3	85		50	Downstream	
85	Sd	48	BHA	0	DP	3	0	38	38	Downstream	
				4.2	DP	3	51			Upstream	
					DP	3	19			Downstream	
					DKP	3	61			Downstream	
86	Bs		NA		D	3	2	40	42		

Table 13. Slit Sampler Data (cont.)

Exp. No.	Spec.	Cult. Age (hrs)	Media	N x10-5	Liquid	n	R	R.H. (%)	Air Temp. (°F)	Notes
86 (cont.)					DP	3	15			
					DKP	3	25			
87	Bs		NA	0.13	TP	3	2.6	37	47	
				0.27		3	5.1			
				0.73		3	12			
				1.5		3	23			
				2.4		3	35			
88	Ea	46	NA	0	TP	3	0	100	44	Downstream (1) EA pour plates.
			NA	3.5		3	8.7			Downstream
						3	17			Upstream
89	Bs		TSA	0.16	DKP	3	12	68	48	
				0.38		3	47			
				1.0		3	96			
				1.6		3	149			
				3.2		3	244			

Table 13. Slit Sampler Data (cont.)

Exp. No.	Spec.	Cult. Age (hrs)	Media	N x10^{-5}	Liquid	n	R	R.H. (%)	Air Temp. (°F)	Notes
90	Bs		NA	0.41	TP	3	4		32	
				1.5		3	16		38	
				3.2		3	26		34	
						3	28		34	Add 50 ml boiled Sm/Lactose broth.
91	Sd		BHA	1.6	DKP	3	13	35	44	
				3.0		3	19			
				4.7		3	24			
				6.6		3	37			
92	Sm	45	NA	0.90	DKP	3	38	54	47	(1) MF water analyses for first 3 sample groups resulted in N's of 0.35, 0.90, and 0.88.
				1.4		3	63			
				1.8		3	69			
				2.4		3	83			
93	Bs		NA	0.48	TP	3	4.3	47	44	
				1.1		3	13	61	49	

Table 13. Slit Sampler Data (cont.)

Exp. No.	Spec.	Cult. Age (hrs)	Media	N x10^{-5}	Liquid	n	R	R.H. (%)	Air Temp. (°F)	Notes
93 (cont.)				2.1		3	27			
				3.0		3	49			
				4.1		3	71	40	58	
94	Sm	43	NA	0.60	TP	3	10	86	43	
				1.4		3	18			
				1.7		3	24			
				2.4		3	35			
				3.7		3	52	47	43	
95	Bs		NA	2.4	TP	3	20	51	39	Downstream
				2.4		3	115	37	42	Upstream
				2.4		3	24	30	47	Downstream
				2.2		3	23			Downstream
				2.1		3	24	31	50	Downstream
96	Sd/Bs		BHA	1.4	TP	3	5	47	60	Sd
				1.9			33			Bs

Table 13. Slit Sampler Data (cont.)

Exp. No.	Spec.	Cult. Age (hrs)	Media	N x10^{-5}	Liquid	n	R	R.H. (%)	Air Temp. (°F)	Notes
96 (cont.)				2.0	TP	3	10			Sd (Aeration liquid changed).
				3.3			79			Bs
				2.3		3	8			Sd (1 hr-45 min. after previous group).
				3.6			96			Bs
97	Sm/Bs		NA	1.1	DK	4	86	34	60	Sm
				0.92			60			Bs
				1.1	DKP	3	88			Sm
				0.94			48			Bs
98	Sm/Bs	41	NA	2.2	TP	4	56	58	48	Sm
				1.8			24			Bs
				3.5	TP	8	97	41	55	Sm (Aeration liquid changed).
				3.6			44			Bs
99	Ss/Bs	42	BHA	0.96	TP	4	14	54	45	Ss
				1.7			29			Bs
				1.8	TP	4	4	41	63	Ss (Aeration liquid changed).

Table 13. Slit Sampler Data (cont.)

Exp. No.	Spec.	Cult. Age (hrs)	Media	N x10^{-5}	Liquid	n	R	R.H. (%)	Air Temp. (°F)	Notes
99 (cont.)				3.4		4	89			Bs
100	Bs		NA	4.2	TP	1	5.7	69	73	V = 3.5 fps (1) No. aerosols/ ft^3 is given as R.
						1	3.5			V = 13.9 fps
						1	4.0			V = 13.2 fps
						1	4.6	100		V = 4.6 fps
						1	2.5			V = 5.4 fps
						1	3.8			V = 6.2 fps
						1	2.7			V = 9.3 fps
						1	3.3			V = 3.0 fps
						1	4.0	90	67	V = 10.3 fps
						1	5.7			V = 3.9 fps
						1	5.1			V = 6.8 fps
						1	4.7			V = 6.7 fps
						1	6.7			V = 4.6 fps
						1	5.9	100		V = 3.0 fps

Table 13. Slit Sampler Data (cont.)

Exp. No.	Spec.	Cult. Age (hrs)	Media	N x10^{-5}	Liquid	n	R	R.H. (%)	Air Temp. (^{o}F)	Notes
101	Bs		NA	3.3	TP	3	47	64	61	
				5.9		3	101			
				10.2		3	136			
				13.7		3	157			
				15.8		3	161	68	60	
102	Sm/Bs	47	NA	0.85	DKP	4	105	46	81	Sm
				1.1			89			Bs
103	Ss	46	PRS	0.43	TP	3	11	46	49	
				0.90		3	17			
				1.7		3	26		56	
				2.4		3	33			
				3.2		3	53			
104	Bs		NA	3.8	TP	3	14	43	54	#27x1/2" needles (1) Hypo bubbler with needle sizes shown operated at 0.5 lpm.
						3	13	31	58	#26x1/2" needles

Table 13. Slit Sampler Data (cont.)

Exp. No.	Spec.	Cult. Age (hrs)	Media	N x10^{-5}	Liquid	n	R	R.H. (%)	Air Temp. (°F)	Notes
104 (cont.)						3	9.4			#24x1/2" needles
105	Ss	46	PRS	1.2	DKP	3	30	43	58	
				2.4		3	62	50	60	
				3.5		3	111			
				4.4		3	184	52	60	
106	Bs									See Appendix G.
107	Sm/Bs	47	NA	0.88	T	3	226	74	54	Sm
				0.99			51			Bs
				1.8		6	29	77	57	Sm (5 mg/l Ivory soap added).
				2.0			6.4			Bs
108	Bs		NA	3.4	T	3	68	32	46	No Tide.
				3.5		3	21			2 mg/l Tide.
						3	16			5 mg/l Tide.
						3	8.6			10 mg/l Tide.

Table 13. Slit Sampler Data (cont.)

Exp. No.	Spec.	Cult. Age (hrs)	Media	N x10^{-5}	Liquid	n	R	R.H. (%)	Air Temp. (°F)	Notes
109	Bs		NA		TP	4	138	46	78	(1) Small aeration tank. (2) N calculated as 2.1x10^{5}.
110	Ss/Bs		BHA	1.9	Sew.	4	7.3			Ss (1) Small aeration tank. (2) Autoclaved, settled
				2.4			19			Bs sewage.
111	Sm/Bs	44	NA	0.92	Sew.	4	32	60	79	Sm (1) Small aeration tank. (2) Autoclaved, settled
				1.5			17			Bs sewage.
112	Sm/Bs	45	NA	1.7	T	4	80	62	39	Sm
				1.8			24			Bs
					TP	3	48	55	43	Sm
							15			Bs
					T2P	3	204	52	45	Sm
							82			Bs
113	Bs		NA	2.2	TP	3	35	42	52	
						3	50			30 ml BHI added.
						3	69	35	52	60 ml BHI added.

Table 13. Slit Sampler Data (cont.)

Exp. No.	Spec.	Cult. Age (hrs)	Media	N $\times 10^{-5}$	Liquid	n	R	R.H. (%)	Air Temp. (^{o}F)	Notes
113 (cont.)						3	73	48	51	90 ml BHI added.
114	Ss/Bs	49	BHA	4.3	TP	5	36	42	70	Ss
				2.8			108			Bs
115			BHA	5.9	Sew.	4	18	72	74	Natural organisms from settled sewage.
116			BHA	10.0	Sew.	4	16	36	80	Natural organisms from settled sewage.
117	Ea/Bs	42	NA	8.5	TP	4	2	50	52	Ea
				1.9			65			Bs
118	Sd/Bs	50	BHA	2.1	TP	3	17	28	76	Sd
				1.9			64			Bs
						3	14			Sd
							100			Bs
119	Bs		NA	3.2	TP	1	6.5	68	66	V = 3.6 fps (1) No./ft^3 is given as R.
						1	4.1			V = 10.4 fps

Table 13. Slit Sampler Data (cont.)

Exp. No.	Spec.	Cult. Age (hrs)	Media	N x10^{-5}	Liquid	n	R	R.H. (%)	Air Temp. (^{o}F)	Notes
119 (cont.)						1	6.3			V = 5.1 fps
						1	8.8			V = 4.4 fps
						1	5.1			V = 6.9 fps
						1	8.5			V = 3.3 fps
						1	4.9			V = 9.8 fps
						1	10.7			V = 3.6 fps
						1	8.2			V = 4.3 fps
						1	9.5			V = 4.5 fps
						1	7.9			V = 5.3 fps
						1	8.3	42	70	V = 8.0 fps
120	Bs									See Appendix G.
121	Sd/Bs	47	BHA	1.5	DK	3	27	40	72	Sd
				1.7			71			Bs
					DKP	3	31			Sd
							92			Bs

Table 13. Slit Sampler Data (cont.)

Exp. No.	Spec.	Cult. Age (hrs)	Media	N x10^{-5}	Liquid	n	R	R.H. (%)	Air Temp. (°F)		Notes
122	Ss/Bs	43	BHA	1.6	DKP	4	56	69	65	Ss	
				1.0			72			Bs	
123	Sm/Bs	51	NA	2.1	TP	4	125	70	60	Sm	
				2.4			59			Bs	
124	Sm/Bs	47	NA	1.1	TP	4	91	48	77	Sm	
				2.0			78			Bs	
125	Ea/Bs	45	NA	>6.6	Sew.	4	0	37	57	Ea	(1) Autoclaved, settled sewage.
				2.2			18			Bs	

APPENDIX D

ANDERSEN SAMPLER DATA

Table 14 contains the results of all samples taken with the Andersen sampler. The data are presented in a manner similar to that in the preceding appendix except that columns instead of special notes are used for wind velocity and bubbler flow rate, and relative humidity and air temperature are shown as general notes. Total or average colony counts for the time shown under "notes" are given for each sample group and have been corrected for overlapping in accordance with Appendix A.

Table 14. Andersen Sampler Data

Exp. No.	Spec.	Cult. Age (hrs)	No./ml x10^{-5}	Liquid	n	Plate No.	Colony Count	V (fps)	Bubbler Flow (lpm)	Notes
17	Ec	73	1.7	DKP	1	1	0	3.8	0.5	(1) 2-med. bubblers.
						2	0			(2) 5 min. samples.
						3	0			
						4	0			
						5	0			
						6	0			
					1	1	0		1.0	
						2	0			
						3	0			
						4	0			
						5	0			
						6	0			
					1	1	0	3.6	1.5	
						2	0			
						3	0			
						4	0			
						5	0			
						6	0			
18	Sm	49	8.8	DKP	3	1	20	3.9	1.0	(1) 2-coarse bubblers.
						2	96			(2) Rel.humid. = 22%.
						3	322			(3) Air temp. = 75°F.
						4	252			(4) 20 min. samples.
						5	47			
						6	0			

Table 14. Andersen Sampler Data (cont.)

Exp. No.	Spec.	Cult. Age (hrs)	No./ml $x10^{-5}$	Liquid	n	Plate No.	Colony Count	V (fps)	Bubbler Flow (lpm)	Notes
20	Sm	47	0.78	DKP	3	1	43	3.2	1.0	(1) 2 med. bubblers.
						2	262			(2) 15 min. samples.
						3	(3)			(3) Exceeded capacity of
						4	399			plate.
						5	17			
						6	0			
21	Sm	47	1.0	DKP	1	1	5	4.5	1.0	(1) 2 coarse bubblers.
						2	27			(2) 10 min. samples.
						3	51			
						4	42			
						5	0			
						6	0			
			2.0		1	1	9	4.6	1.0	
						2	35			
						3	88			
						4	31			
						5	3			
						6	0			
			4.1		1	1	11	4.9	1.0	
						2	54			
						3	156			
						4	59			
						5	5			
						6	0			

Table 14. Andersen Sampler Data (cont.)

Exp. No.	Spec.	Cult. Age (hrs)	No./ml x10^{-5}	Liquid	n	Plate No.	Colony Count	V (fps)	Bubbler Flow (lpm)	Notes
22	Sm	½-44	3.0	DKP	1	1	31	3.6	0.5	(1) 1 coarse bubbler.
						2	40			(2) 20 min. samples.
		½-70				3	91			(3) Wind velocity varied
						4	41			as shown.
						5	5			(4) Rel. humid. = 17%.
						6	0			(5) Air temp. = 72°F.
					1	1	46	4.9		
						2	46			
						3	103			
						4	37			
						5	2			
						6	0			
					1	1	32	4.9		
						2	31			
						3	97			
						4	50			
						5	2			
						6	0			
					1	1	31	5.3		
						2	46			
						3	120			
						4	42			
						5	0			
						6	0			

Table 14. Andersen Sampler Data (cont.)

Exp. No.	Spec.	Cult. Age (hrs)	No./ml x10^{-5}	Liquid	n	Plate No.	Colony Count	V (fps)	Bubbler Flow (lpm)	Notes
22 (cont.)					1	1	27	8.1		
						2	30			
						3	108			
						4	30			
						5	3			
						6	0			
23	Sm	48	2.7	DKP	1	1	212	4.0	0.5	(1) 1 med. bubbler.
						2	63			(2) 10 min. samples.
						3	285			(3) Wind velocity varied
						4	206			as shown.
						5	23			(4) Rel.humid. = 18%.
						6	1			(5) Air temp. = 62°F.
					1	1	82	4.0		
						2	128			
						3	509			
						4	328			
						5	14			
						6	1			
					1	1	103	5.4		
						2	164			
						3	531			
						4	267			
						5	16			
						6	0			

Table 14. Andersen Sampler Data (cont.)

Exp. No.	Spec.	Cult. Age (hrs)	No./ml x10^{-5}	Liquid	n	Plate No.	Colony Count	V (fps)	Bubbler Flow (lpm)	Notes
23 (cont.)					1	1	121	6.5		
						2	196			
						3	649			
						4	239			
						5	32			
						6	0			
					1	1	98	8.1		
						2	165			
						3	597			
						4	236			
						5	17			
						6	6			
25	Sm	72	0.48	DKP	1	1	19	3.8	0.5	(1) 1 med. bubbler.
						2	25			(2) 15 min. samples.
						3	50			
						4	26			
						5	2			
						6	0			
			1.0		1	1	34	3.7		
						2	54			
						3	129			
						4	32			
						5	3			
						6	0			

Table 14. Andersen Sampler Data (cont.)

Exp. No.	Spec.	Cult. Age (hrs)	No./ml x10^{-5}	Liquid	n	Plate No.	Colony Count	V (fps)	Bubbler Flow (lpm)	Notes
25 (cont.)			2.2		1	1	38	3.7		
						2	81			
						3	267			
						4	130			
						5	9			
						6	0			
			3.9		1	1	91			
						2	166			
						3	520			
						4	271			
						5	11			
						6	0			
			8.3		1	1	60	4.3		
						2	190			
						3	680			
						4	326			
						5	23			
						6	1			
26	Sm		0.55	T	1	1	0	4.0	0.5	(1) 1 med. bubbler for 1st and 4th samples and 1 coarse for 2nd and 3rd samples. (2) Sampling times 10, 20, 15 and 10 min., respectively. (3) Air temp. = 56°F.
						2	0			
						3	3			
						4	1			
						5	2			
						6	1			

Table 14. Andersen Sampler Data (cont.)

Exp. No.	Spec.	Cult. Age (hrs)	No./ml x10^{-5}	Liquid	n	Plate No.	Colony Count	V (fps)	Bubbler Flow (lpm)		Notes
26 (cont.)			10.0		1	1	0	4.5			
						2	0				
						3	2				
						4	1				
						5	1				
						6	0				
					1	1	2	4.4			
						2	2				
						3	10				
						4	15				
						5	5				
						6	3				
					1	1	2	4.4			
						2	1				
						3	9				
						4	22				
						5	11				
						6	0				
28	Sm	72	7.6	DKP	1	1	35	3.8	0.5	H=1-1/2"	(1) Rel.humid.=59%.
						2	37				(2) 1 med.bubbler.
						3	190				(3) Sampling times
						4	216				are 10,10,15,21
						5	15				and 20, respectively.
						6	0				

Table 14. Andersen Sampler Data (cont.)

Exp. No.	Spec.	Cult. Age (hrs)	No./ml x10^{-5}	Liquid	n	Plate No.	Colony Count	V (fps)	Bubbler Flow (lpm)	Notes
28 (cont.)					1	1	22	3.3		H=4-3/4".
						2	24			
						3	120			
						4	67			
						5	6			
						6	0			
					1	1	17	3.6		H=8-5/8".
						2	17			
						3	67			
						4	50			
						5	4			
						6	0			
					1	1	3	4.1		H=11-3/8".
						2	2			
						3	5			
						4	4			
						5	0			
						6	0			
					1	1	4	3.4		H=16-5/8".
						2	0			
						3	1			
						4	1			
						5	0			
						6	0			

Table 14. Andersen Sampler Data (cont.)

Exp. No.	Spec.	Cult. Age (hrs)	No./ml x10^{-5}	Liquid	n	Plate No.	Colony Count	V (fps)	Bubbler Flow (lpm)	Notes
30	Sm	73	5.7	DKP	1	1	29	2.9	0.5	H=0" (1) Rel.humid.=54%.
						2	83			(2) Air temp.=61°F.
						3	237			(3) 1 med.bubbler.
						4	102			(4) 10 min.samples.
						5	10			
						6	0			
					1	1	67	3.6		H=1-3/4"
						2	44			
						3	241			
						4	204			
						5	18			
						6	0			
					1	1	14	2.1		H=4-5/8"
						2	14			
						3	56			
						4	36			
						5	4			
						6	0			
					1	1	1	4.0		H=8-1/2"
						2	0			
						3	5			
						4	5			
						5	0			
						6	0			

Table 14. Andersen Sampler Data (cont.)

Exp. No.	Spec.	Cult. Age (hrs)	No./ml x10^{-5}	Liquid	n	Plate No.	Colony Count	V (fps)	Bubbler Flow (lpm)	Notes
30 (cont.)					1	1	0	3.9		H=12-7/8"
						2	0			
						3	0			
						4	1			
						5	0			
						6	0			
31	Sm	47	6.0	DKP	1	1	79	5.8	0.5	H=0" (1) Rel.humid.=61%.
						2	307			(2) Air temp.=55°F.
						3	1125			(3) 1 med. bubbler.
						4	499			(4) 15 min. samples.
						5	86			
						6	2			
					1	1	48	5.6		H=6-1/4"
						2	62			
						3	166			
						4	65			
						5	7			
						6	0			
					1	1	164			H=1-3/8"
						2	241			
						3	963			
						4	478			
						5	47			
						6	2			

Table 14. Andersen Sampler Data (cont.)

Exp. No.	Spec.	Cult. Age (hrs)	No./ml x10^{-5}	Liquid	n	Plate No.	Colony Count	V (fps)	Bubbler Flow (lpm)	Notes
33	Sm	46	7.0	DKP	5	1	71	4.6	0.5	(1) Rel.humid.=61%.
						2	80			(2) Air temp.=55°F.
						3	361			(3) 1 med. bubbler.
						4	245			(4) 15 min. samples.
						5	27			
						6	0			
36	Sm	48	0.91	DKP	1	1	55	4.1	0.5	(1) Rel.humid.=61%.
						2	81			(2) 1 med. bubbler.
						3	183			(3) 15 min. samples.
						4	57			(4) 1 mg/l Antifoam B
						5	4			added between samples.
						6	0			
					1	1	15	4.6		
						2	19			
						3	129			
						4	193			
						5	13			
						6	0			
44	Sm	47	0.22	T	1	1	5	4.4	0.5	t=0 hrs (1) Rel.humid.varied
						2	3			from 58 to 82%.
						3	11			(2) Air temp.varied
						4	33			from 59 to 64°F.
						5	17			(3) 1 med. bubbler.
						6	0			(4) 15 min.samples.

Table 14. Andersen Sampler Data (cont.)

Exp. No.	Spec.	Cult. Age (hrs)	No./ml x10^{-5}	Liquid	n	Plate No.	Colony Count	V (fps)	Bubbler Flow (lpm)	Notes
44 (cont.)					1	1	0	4.1		t=1.15 hrs.
						2	3			
						3	12			
						4	33			
						5	21			
						6	0			
					1	1	3	3.6		t=2.42 hrs.
						2	1			
						3	13			
						4	24			
						5	26			
						6	2			
					1	1	1	4.5		t=3.67 hrs.
						2	2			
						3	13			
						4	29			
						5	32			
						6	0			
					1	1	0	3.6		t=5.38 hrs.
						2	1			
						3	12			
						4	27			
						5	19			
						6	0			

Table 14. Andersen Sampler Data (cont.)

Exp. No.	Spec.	Cult. Age (hrs)	No./ml x10^{-5}	Liquid	n	Plate No.	Colony Count	V (fps)	Bubbler Flow (lpm)	Notes
45	Sm	48	0.22	T 0.1P	1	1	9	4.9	0.5	t=0 hrs. (1) Rel.humid. varied from 45 to 50%. (2) Air temp.=62°F. (3) 1 med.bubbler. (4) 15 min.samples.
						2	3			
						3	34			
						4	51			
						5	18			
						6	1			
					1	1	4	4.4		t=1.18 hrs.
						2	0			
						3	21			
						4	25			
						5	16			
						6	0			
					1	1	4	4.4		t=2.57 hrs.
						2	4			
						3	12			
						4	26			
						5	15			
						6	1			
					1	1	4	3.7		t=4.08 hrs.
						2	0			
						3	11			
						4	27			
						5	16			
						6	0			

Table 14. Andersen Sampler Data (cont.)

Exp. No.	Spec.	Cult. Age (hrs)	No./ml x10^{-5}	Liquid	n	Plate No.	Colony Count	V (fps)	Bubbler Flow (lpm)	Notes
45 (cont.)					1	1	2			t=4.43 hrs.
						2	0			
						3	9			
						4	38			
						5	9			
						6	2			
46	Sm	45	0.30	TP	1	1	32	3.8	0.5	t=0 (1) Rel.humid. varied
						2	38			from 58 to 79%.
						3	65			(2) Air temp. varied
						4	24			from 61 to 68°F.
						5	2			(3) 1 med. bubbler.
						6	1			(4) 15 min. samples.
					1	1	26			t=1.78 hrs.
						2	32			
						3	52			
						4	16			
						5	0			
						6	0			
					1	1	11	4.1		t=4.03 hrs.
						2	14			
						3	21			
						4	8			
						5	1			
						6	0			

Table 14. Andersen Sampler Data (cont.)

Exp. No.	Spec.	Cult. Age (hrs)	No./ml $\times 10^{-5}$	Liquid	n	Plate No.	Colony Count	V (fps)	Bubbler Flow (lpm)	Notes
46 (cont.)					1	1	5	3.8		t=5.82 hrs.
						2	4			
						3	21			
						4	8			
						5	0			
						6	0			
					1	1	15			t=9.18 hrs.
						2	10			
						3	17			
						4	6			
						5	1			
						6	0			
50	Sm	47	(1)	TP	1	1	4	3.8	0.5	t=0 (1) See Table 13, Exp. 50 for water analyses. (2) Rel.humid=77%. (3) Air temp.=53°F. (4) 15 min. samples. (5) 1 med. bubbler.
						2	11			
						3	28			
						4	12			
						5	0			
						6	0			
					1	1	9	3.8		t=1.20 hrs.
						2	9			
						3	24			
						4	12			
						5	2			
						6	0			

Table 14. Andersen Sampler Data (cont.)

Exp. No.	Spec.	Cult. Age (hrs)	No./ml x10^{-5}	Liquid	n	Plate No.	Colony Count	V (fps)	Bubbler Flow (lpm)	Notes
50 (cont.)					1	1	2	2.9		t=3.03 hrs.
						2	6			
						3	12			
						4	3			
						5	0			
						6	0			
					1	1	5	4.1		t=5.28 hrs.
						2	2			
						3	7			
						4	6			
						5	0			
						6	0			
					1	1	3			t=25.7 hrs.
						2	1			
						3	7			
						4	0			
						5	1			
						6	0			
80	Aa	45	6.0	DKP	3	1	8	3.8	0.5	(1) Upstream sampling point. (2) Rel.humid.=49%. (3) Air temp.=46°F. (4) 15 min. samples. (5) 1 med. bubbler.
						2	7			
						3	8			
						4	1			
						5	1			
						6	0			

Table 14. Andersen Sampler Data (cont.)

Exp. No.	Spec.	Cult. Age (hrs)	No./ml x10^{-5}	Liquid	n	Plate No.	Colony Count	V (fps)	Bubbler Flow (lpm)	Notes
81	Bs		0.13	TP	3	1	7	2.6	0.5	(1) Rel.humid.=37%.
						2	3			(2) Air temp.=47°F.
						3	2			(3) 15 min. samples.
						4	0			(4) 1 med. bubbler.
						5	3			
						6	1			
			0.73		3	1	8	1.8		
						2	5			
						3	7			
						4	2			
						5	2			
						6	1			
			2.4		3	1	15	1.6		
						2	14			
						3	27			
						4	13			
						5	2			
						6	0			
89	Bs		0.38	DKP	3	1	8	3.7	0.5	(1) Rel.humid.varied from
						2	13			58 to 68%.
						3	24			(2) Air temp. varied from
						4	22			48 to 55°F.
						5	1			(3) 15 min. samples.
						6	0			(4) 1 med. bubbler.

Table 14. Andersen Sampler Data (cont.)

Exp. No.	Spec.	Cult. Age (hrs)	No./ml x10^{-5}	Liquid	n	Plate No.	Colony Count	V (fps)	Bubbler Flow (lpm)	Notes
89 (cont.)			1.0		3	1	25	3.5		
						2	25			
						3	60			
						4	39			
						5	5			
						6	1			
			3.2		3	1	60	3.6		
						2	60			
						3	192			
						4	157			
						5	24			
						6	0			
90	Bs		1.5	TP	3	1	5	3.6	0.5	(1) Air temp. varied from 32 to 38°F.
						2	3			(2) 15 min. samples.
						3	7			(3) 1 med. bubbler.
						4	4			
						5	1			
						6	0			
			3.2		3	1	9	3.9		
						2	10			
						3	28			
						4	13			
						5	1			
						6	0			

Table 14. Andersen Sampler Data (cont.)

Exp. No.	Spec.	Cult. Age (hrs)	No./ml x10^{-5}	Liquid	n	Plate No.	Colony Count	V (fps)	Bubbler Flow (lpm)	Notes
104	Bs		3.8	TP	3	1	3	3.8	0.5	(1) Rel.humid. varied from 31 to 43%.
						2	3			(2) Air temp. varied from 54 to 58°F.
						3	13			(3) 15 min. samples.
						4	6			(4) Hypo bubbler-needle sizes used were #27, #26, and #24, respectively for the 3 sample groups.
						5	7			
						6	1			
					3	1	7	3.8		
						2	9			
						3	11			
						4	5			
						5	1			
						6	0			
					3	1	1	3.8		
						2	2			
						3	5			
						4	1			
						5	1			
						6	0			
112	Bs/Sm	45	1.8	TP	3	1	88	4.2	0.5	Bs (1) Rel.humid.varied from 52 to 62%.
						2	84			(2) Air temp.varied from 39 to 45°F.
						3	124			(3) 15 min. samples.
						4	31			(4) 1 med. bubbler.
						5	3			(5) 3 samples with Sm and Bs counts as shown.
						6	1			

Table 14. Andersen Sampler Data (cont.)

Exp. No.	Spec.	Cult. Age (hrs)	No./ml x10^{-5}	Liquid	n	Plate No.	Colony Count	V (fps)	Bubbler Flow (lpm)	Notes
112 (cont.)			1.7			1	198			Sm
						2	176			
						3	302			
						4	83			
						5	3			
						6	0			

APPENDIX E

COEFFICIENT OF VARIATION OF SLIT SAMPLERS

Table 15. Data Used in the Determination of the Coefficient of Variation of Slit Samples

Run No.	Species	n	C_V(%)	N_S
95	Bs	12	21	52
63	Sm	9	24	116
36	Sm	5	3.9	409
36	Sm	5	5.7	458
117	Bs	4	5.2	153
114	Bs	5	6.7	252
114	Ss	5	18	83
122	Bs	4	12	169
122	Ss	4	10	130
99	Bs	4	7.4	68
99	Ss	4	17	32
124	Bs	4	11	183
124	Sm	4	5.2	213
98	Bs	4	12	56
98	Sm	4	5.6	130
98	Bs	8	12	104
98	Sm	8	8.8	227
123	Bs	4	10	137
123	Sm	4	14	291
97	Bs	4	4.4	141
97	Sm	4	16	201
96	Bs	6	17	204

Table 15. Data Used in the Determination of the Coefficient of Variation of Slit Samples (cont)

Run No.	Species	n	C_V(%)	N_S
110	Bs	4	32	44
110	Ss	4	21	17
125	Bs	4	27	41
116	Sewage	4	16	38
115	Sewage	4	20	42
109	Bs	4	5.6	322
111	Bs	4	8.8	40

APPENDIX F

SLIT VERSUS ANDERSEN SAMPLERS

Table 16. Comparison of Slit and Andersen Samplers

Exp. No.	n_{Slit}	$n_{And.}$	$(R)_s/(R)_A$
89	9	9	1.58
90	6	6	1.44
87	6	6	1.13
104	6	6	0.96
46	15	5	0.98
50	12	4	1.28
33	15	5	1.28
44	15	5	1.36
45	12	5	0.92
21	8	3	0.85

$\sum (R)_s/(R)_A = 11.78$

$\left[(R)_s/(R)_A\right]_{ave.} = 1.18$

$\sigma = 0.25$

$t_{\alpha,n-1} = (1.18-1.00)\ \frac{\sqrt{n}}{\sigma} = \frac{0.18\sqrt{10}}{0.25} = 2.28$

$\alpha = 0.025$

APPENDIX G

DISTRIBUTION OF AEROSOLS AT DOWNSTREAM POINT

EXPERIMENT NO. 106

Species - B. subtilis
Liquid - TP
Bubbler - Medium No. 4
Intake - 22mm diameter

Media - NA
Sampler - slit
R.H. - 50 to 57%
Air temp. - 48 to 62°F

H* (inches)	P** (inches)	Number Samples	No./ft^3
2 - 1/4	9 L	2	18.9
"	4-1/2 L	3	15.3
"	0	3	12.7
"	4-1/2 R	3	11.0
"	9 R	2	8.3
6	9 L	2	8.3
"	4-1/2 L	3	5.4
"	0	3	8.6
"	4-1/2 R	3	4.1
"	9 R	2	4.3
10-1/4	4-1/2 L	2	3.8
"	0	2	3.7
"	4-1/2 R	2	1.9
12-3/8	0	1	3.3

*H indicates the height of the sample intake above the floor of the wind tunnel.

**P indicates the distance to the left or right of the centerline of the tunnel when looking upstream.

EXPERIMENT NO. 120

Species - B. subtilis
Liquid - TP
Bubbler - medium No. 4
Intake - 22mm diameter

Media - NA
Sampler - slit
R.H. - 26 to 47%
Air temp. - 40 to 55°F

H* (inches)	P** (inches)	Number Samples	No./ft^3
2-1/4	9 L	2	18.4
"	4-1/2 L	2	11.5
"	0	3	10.3
"	4-1/2 R	2	7.7
"	9 R	2	6.1
5	4-1/2 L	2	7.3
"	4-1/2 R	2	3.3
8	4-1/2 L	2	5.2
"	4-1/2 R	2	1.2
11	4-1/2 L	2	3.7
"	4-1/2 R	2	0.7
14	0	3	2.1

*H indicates the height of the sample intake above the floor of the wind tunnel.
**P indicates the distance to the left or right of the centerline of the tunnel when looking upstream.

Table 17. Vertical Distribution of Aerosols

Exp. No.	H (in.)	C*	$\frac{C}{C_o}$ **
106	2.25	13.3	1.05
106	6.00	6.2	0.49
106	10.25	3.1	0.24
106	12.38	3.3	0.26
120	2.25	10.8	1.05
120	5.00	5.3	0.52
120	8.00	3.3	0.32
120	11.00	2.2	0.21
120	14.00	2.1	0.20

*Average No. aerosols/ft3 at elevation shown.
**C_o is No. aerosols/ft^3 at the centerline and H=2.25".

APPENDIX H

TYPICAL ANALYSIS OF ATLANTA TAP WATER*

Silica (SiO_2)	9.5 mg/l
Alkalinity (P.)	3.0 mg/l
Alkalinity (M.O.)	16.0 mg/l
Chlorine Residual	1.2 mg/l
Carbon Dioxide (CO_2)	0.00 mg/l
Color	5.00
Dissolved Oxygen	97.00%
Dissolved Solids (Conductivity)	30.00 mg/l
Hardness ($CaCO_3$)	22.0 mg/l
Iron (Fe)	0.02 mg/l
Sulphates (SO_4)	4.00 mg/l
pH (Colorimetric)	8.6
Alumina (Al)	0.05 mg/l
Chloride (Cl)	4.00 mg/l
Turbidity	0.10 mg/l
Calcium (Ca)	7.1 mg/l
Magnesium (Mg)	1.0 mg/l
Manganese (Mn)	0.02 mg/l
Carbonate (CO_3)	3.6 mg/l
Bicarbonate (HCO_3)	12.2 mg/l

*From: J. L. Carr, Supervisor, Hemphill Laboratory, Water Treatment Division, Department of Water Works, Atlanta, Georgia.

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